Behavioural effects of melatonin with reference to the serotonergic system and circadian rhythms

Pache, D. M.

Award date:
1990

Awarding institution:
University of Bath

BEHAVIOURAL EFFECTS OF MELATONIN WITH REFERENCE TO THE SEROTONERGIC SYSTEM AND CIRCADIAN RHYTHMS

Submitted by D. M. Pache B.Pharm., M.R.Pharm.S.

for the degree of

Doctor of Philosophy

of the

University of Bath

1990

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

To Mum and Dad

ACKNOWLEDGEMENTS

I would like to express my thanks to Dr. P.H. Redfern for the continual support and encouragement he has provided throughout this project. I would also like to thank Professor R.J. Flower for the use of the facilities of the Department of Pharmacology.

I would like to thank Dr. J.F. Stolz for his support and encouragement during this project and his expert help in the field of computer programming.

The technical assistance of Mrs M. Norris and her staff at the University of Bath Animal House (especially Ewen, Leslie and Mike) was particularly appreciated. I would also like to extend thanks to Ms Ria Baguste and her staff at the Department of Pharmacology (especially Gordon and Dave) for their invaluable technical assistance.

I would like to give special thanks to Mike Harriman of the Department of Physics for his expert help in the field of computer interfacing, and Dr. Paul Christie for his expert help in the field of statistics.

I would like to take this opportunity to thank Helen, Helen and Sue for their secretarial assistance in typing the figures.

I would like to express my gratitude to Ashish Singh for his part in our "scholarly discussions".

My warmest thanks go to my parents and my sister for their love and support, and to Alison, my partner, for her love and understanding throughout. Without them this work would not have been possible.

CONTENTS

SUMMARY

The present study set out to investigate whether melatonin interacts with the serotonergic system. Established rodent behavioural models for specific 5-hydroxytryptamine (5-HT) mediated behaviour were used to test this hypothesis. The generation of headtwitches by the non-specific 5-HT agonists 5-Methoxy-N,N-dimethyltryptamine (5MeODMT) and 5-hydroxy-L-tryptophan (L-5HTP) was used as a model for the activition of 5-HT_2 receptors; hyperphagia and hypothermia induced by 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT) were used as models for 5-HT_{1A} receptor activation; and hyperactivity induced by 5-methoxy-3-(1,2,3,6-tetrahydropyrindin-4-yl)-1H-indole (RU24969) was used as a putative model for 5-HT_{1B} receptor activation. It was recognized that sensitivity to melatonin might vary over 24 h and therefore experiments were conducted at four time points where conditions permitted. Melatonin had no effect on any of the behavioural models at any of the time points investigated.

The discriminative stimulus produced by L-5HTP was used as a further example of 5-HT receptor activation. Melatonin was found to have no effect on the cue generated by L-5HTP using the T-maze (shock escape) drug discrimination paradigm. Furthermore, melatonin itself failed to produce a discriminative stimulus that could be distinguished from saline using both the T-maze and Skinner box (food reward) drug discrimination paradigms at doses upto 50mg/kg ip. Moreover, melatonin was found to be without effect on both the visual and temporal components of a combined light-dark:time-of-day discriminative cue.

A second series of experiments examined the effects of

melatonin on the circadian rhythm of locomotor activity in rats. Melatonin failed to affect the rate and direction of re-entrainment following a phase inversion of the LD cycle. However, the ability of appropriately-timed injections of melatonin to entrain free-running circadian locomotor activity rhythms was confirmed. Since melatonin does not act as a discriminative stimulus at similar doses it is concluded that this ability is not dependent upon the periodic generation of a cognitive signal that can be recognized by the animal.

CHAPTER 1.

INTRODUCTION

1. INTRODUCTION

It is the intention of this introduction to give an overview of the three main subjects covered in this thesis, namely melatonin, circadian rhythms and 5-hydroxytryptamine (5-HT). Melatonin is considered first; its site of manufacture, its synthesis, its physiological and pharmacological properties and the identification of specific melatonin receptors. There follows a section describing the properties and regulation of circadian rhythms. The final part of this chapter is devoted to the biochemistry and physiology of 5-HT. It also discusses the recent proliferation in 5-HT receptor subtypes and their biochemical and behavioural correlates. Specific 5-HT mediated behaviours are examined in greater detail in chapters 3 and 4.

1.1. Melatonin

1.1.1. Morphology and Vascularization of the Mammalian Pineal Gland

The mammalian pineal gland is the major manufacturer of the indoleamine, melatonin. The organ is located in the midline of the brain at the junction of the cerebellum and the cerebral hemispheres. Although it is generally thought of as lying outside the blood brain barrier (Reiter, 1988; Moller et al., 1978) it is connected to the brain via a stalk that descends downwards and attaches to the choroid plexus. Goldman and Wurtman (1964) reported that the gland supports a very generous blood supply, second only to the kidney per gram of tissue. Arterial supply is derived from the posterior choroidal vessels which in turn arise from the posterior cerebral arteries as they travel around the dorsolateral aspect of

the mesencephalon (Reiter, 1981). The pineal parenchyma consists of several cell types commonly found elsewhere e.g. glial cells and mast cells. The pinealocyte, however, is specific to the gland and constitutes the melatonin production factory. It is thought to be derived phylogenically from retinal photoreceptors since lower vertebrates possess modified photoreceptors that bear a strong functional and structural resemblence to their retinal counterparts (Falcon and Collin, 1989). These early pinealocytes are able to detect the presence or absence of light directly unlike those present in mammals that respond to a retinally-derived neural signal only. Structurally, mammalian pinealocytes resemble neurons in that many long cytoplasmic processes spread out from the cell body in various directions. These processes have been shown to terminate near perivascular spaces, blood vessels, calcicular concretions, between connective tissue and between pinealocytes (Vollrath, 1984). One of the most interesting infrastructural features of pinealocytes is the synaptic ribbons and synaptic spherules described by Vollrath (1984). It has been shown that these display an inverse day/night rhythmicity relationship in the guinea pig (Vollrath et al., 1983). Their numbers are low during the daytime, but high at night. It has been suggested by King and Dougherty (1982a, 1982b) that the synaptic ribbons may be involved in the regulation of beta-adrenoceptors located on the pinealocyte membrane.

It is generally accepted now that mammalian pinealocytes are secretory cells that release melatonin into the capillary vessels that vascularize the gland via the capillary spaces and the fenestrated capillary endothelial wall and that this constitutes the major route of secretion (Hedlund et al. 1977; Rollag et al., 1978; Reiter, 1986). From these vessels the product is ultimately able to

drain into the large intracranial veins (Kappers et al., 1973). It has also been suggested by many authors that pinealocytes present in the pineal recess (the stalk extension of the pineal gland that attaches to the third ventricle of the brain at the choroid plexus) secrete melatonin directly into the cerebro-spinal fluid (CSF) (Anton-Tay and Wurtman, 1969; Collu et al., 1971). The pineal recess is described by Hewing (1978) as having an interruption in the ependymal lining which allows pinealocytes to come into direct contact with the CSF. Circumstantial evidence supporting this concept is provided by Hedlund et al. (1977) who identified a higher concentration of melatonin in the CSF of sheep than in the plasma. Conversely, Brown et al. (1979) have shown that human blood titres of melatonin are greater than those measured in the CSF.

A curious feature of the pineal gland is the calcicular concretions which provide a useful radiological landmark for identifying the midsagittal plane of the brain (Vollrath, 1984) but have so far escaped a proven physiological role. It has been shown that calcification increases with age (Kitay and Altschule, 1954; Reiter et al., 1976) and may well reflect the secretory activity of the gland (Vollrath, 1984). This speculation arises from Reiter et al. (1976) who observed a decrease in concretion formation after sympathetic denervation which suppresses melatonin production.

1.1.2. Innervation of the Mammalian Pineal Gland

The ability of light to regulate the synthesis of melatonin was first reported by Wurtman et al. in 1963. This was followed up in 1964 by the same research group who identified the central role played by the sympathetic nervous system in mediating this effect of light (Wurtman et al., 1964a). Since then extensive

studies by Moore and Lenn (1972) and Moore (1973) have established the presence of the retino-hypothalamic tract (RHT), a monosynaptic neural pathway from retina to the suprachiasmatic nucleus (SCN), in addition to the subsequent route to the pineal gland itself. The ganglion cell layer of the retina conveys neurally coded photic information bilaterally to the optic chiasm and, following decussation, to the region of the hypothalamus known as the SCN. From the SCN photic signals are transmitted to the paraventricular nucleus (PVN), also of the hypothalamus, over the medial forebrain bundle to the upper thoracic interiomediolateral cell column of the spinal cord (Klein et al., 1983). Synaptic connections are made at this point with preganglionic cell bodies which innervate the superior cervical ganglia (SCG) whose postganglionic fibres project back to the brain and enter the pineal gland via the conarian nerve. In the rat, at least, it appears that these sympathetic fibres in the pineal gland do not form distinguishable synapses with the pinealocytes, but instead release transmitter into the perivascular space (Moore, 1978). In addition to this peripheral sympathetic innervation, certain species also possess a central innervation from the habenular nuclei that penetrates the pineal organ via the stalk, though its function remains obscure (Korf and Moller, 1984; Dafny, 1980). It is thought that there may be two types of transmission for photic stimuli; a fast one using the central habenulo-pineal route and a slow one via the SCG (Kappers, 1983).

Parasympathetic innervation of the pineal gland has also been reported (Kenny, 1961; Romijn, 1973) along with the identification of a low density of postsynaptic muscarinic binding sites (Taylor et al., 1980). Schrier and Klein (1974) reported an absence of choline acetyltransferase in the gland and Romijn (1975,

1976) observed only minor changes in the infrastructure of pinealocytes after parasympathectomy and administration of parasympatholytic drugs. However, the recent work of Laitinen et al. (1989) suggests that muscarinic M_1 receptors might be involved in phosphoinositide turnover in the rat pineal.

1.1.3. Sources of Melatonin

A number of studies have been published now that counter the early belief that the manufacture of melatonin was the sole prerogative of the pineal organ. The retina (Quay, 1965; Cardinali and Rosner, 1971; Leino and Airaksinen, 1985; Weichmann, 1986), Harderian gland (Bubenik et al., 1974, 1978; Reiter et al., 1981; Hoffmann et al., 1985), the gastrointestinal tract (Raikhlin et al., 1975; Quay and Ma, 1976), the central nervous system (Bubenik et al., 1974), red blood cells (Rosengarten et al., 1972) and blood platelets (Launay et al., 1983) have all been claimed to produce melatonin. As with the pineal organ, production of melatonin in the Harderian gland and the retina exhibits a circadian rhythm (Binkley et al., 1979; Hamm and Menaker, 1980; Pang et al., 1980; Reiter et al., 1983). However, the purpose of this production in these structures has yet to be identified since their contribution to circulating melatonin is minimal. It is important, though, to realize that pinealectomy does not render an animal "melatonin-free" (Reiter et al., 1983).

1.1.4. Biosynthesis of Melatonin

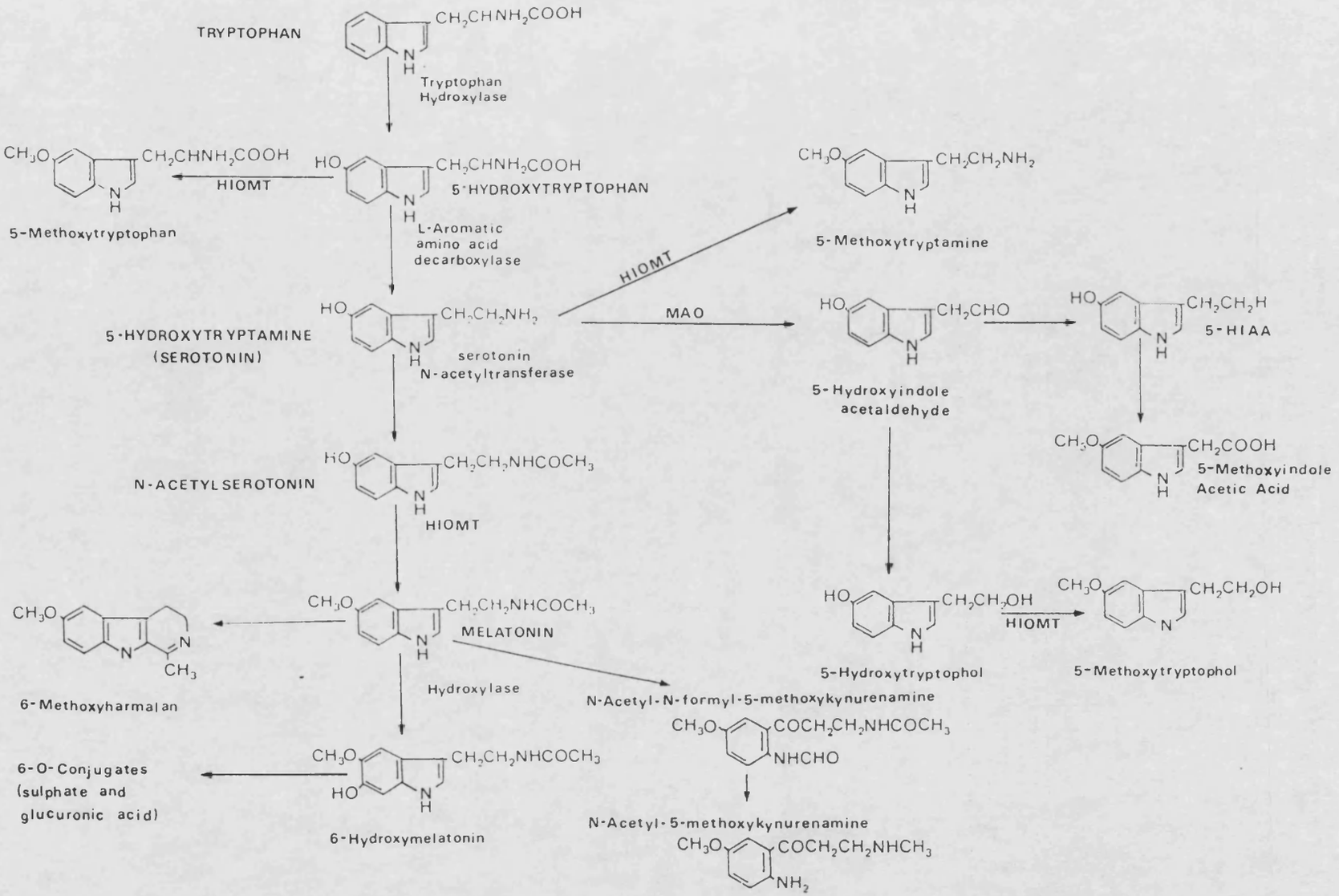
If one ignores the legendary role assigned by philosophers and early anatomists to the pineal gland, McCord and Allen in 1917 were the first to observe a specific action attributable to the

pineal. They demonstrated that an extract of bovine pineal gland caused blanching of the skin of a tadpole. Lerner et al. (1958, 1959) finally identified the active principle as being 5-methoxy-N-acetyltryptamine, which is now known as melatonin. Once discovered the biosynthetic pathways leading up to melatonin production and its subsequent degradation were quickly elucidated (see Fig. 1). Tryptophan is the starting material and this is metabolized to the intermediate compound in the synthetic sequence, 5-HT. 5-HT is then converted into ultimately four products: melatonin, 5-methoxy-tryptamine, 5-methoxyindoleacetic acid or 5-methoxytryptophol. Metabolic elimination of melatonin itself involves hydroxylation to 6-hydroxymelatonin and then conjugation with glucuronic acid in the liver. In the brain melatonin may also be metabolized by cleavage of its indole nucleus into N-gamma-acetyl-N-2-formyl-5-methoxy-kynurenamine and N-gamma-acetyl-5-methoxykynurenamine (Hirata et al., 1974) (See Fig. 1). It has even been suggested that the indoleamines melatonin, serotonin and 5-methoxytryptamine may undergo cyclohydrogenation into psychoactive beta-carboline derivatives (Binkley, 1988).

From its precursor there are four enzymes involved in the synthetic pathway to melatonin production. The first, tryptophan 5-hydroxylase (EC 1.14.16.4, L-tryptophan, tetrahydropteridine:oxygen oxido-reductase (5-hydroxylating)) is found only in serotonin-synthesizing cells and catalizes the formation of 5-hydroxy-L-tryptophan (L-5HTP). The ubiquitously distributed L-aromatic acid decarboxylase (EC 4.1.1.28), located in the pinealocyte cytoplasm, is a pyridoxal-dependent enzyme that rapidly acts upon L-5HTP to produce the optically inactive serotonin. The third enzyme in the sequence is one of two N-acetyltransferases that exist in the pineal

(Voisin et al., 1984). Serotonin arylalkylamine N-acetyl-transferase is the one commonly abbreviated to SNAT and, using acetyl co-enzyme A, preferentially acetylates indoleamines (e.g. tryptamine, 5-HT and 5-methoxytryptamine) rather than phenylethylamines. The second, pineal arylamine N-acetyltransferase, is thought to bear greater homology to extrapineal N-acetyltransferases located in the the blood, liver, thyroid, spleen and a host of other sites (Binkley, 1988) and is not involved in the synthesis of melatonin. The activity of SNAT, believed to reside in a 10kDa portion of the protein (Sugden, 1989), converts serotonin into N-acetylserotonin. Then, using S-adenosyl methionine (SAM) as the donor of the methyl group, hydroxyindole-O-methyltransferase (HIOMT; S-adenosyl-L-methionine: N-acetylserotonin-O-methyltransferase, E.C.2.1.1.4) changes the hydroxyl group on the 5-position of the indole ring to a methoxy group thereby creating 5-methoxy-N-acetyltryptamine (melatonin). This final enzyme shares with SNAT the property of a restricted distribution, being found almost exclusively in the pineal gland. The retina has been shown to possess a much smaller concentration (Axelrod and Weissbach, 1960; Axelrod and Weissbach, 1961), and the Harderian gland is thought to possess an enzyme unrelated to pineal HIOMT (Cardinali and Wurtman, 1972). Kuwano and Takahashi (1980) purified pineal HIOMT and proposed that it consisted of two identical subunits of 38kDa.

Fig. 1. Synthesis and main metabolic routes of melatonin. (Adapted from Ebadi, 1984 and Hirata et al, 1974).



1.1.5. Biosynthetic Factors Influencing Melatonin Production.

The concentration of the essential amino acid tryptophan in the rat pineal gland is high (Mefford et al., 1983; Young and Anderson, 1982) and is thought to enter via an analogous mechanism to that used by the brain for extracting tryptophan from the plasma. Interestingly though the diurnal rhythm expressed by plasma tryptophan, which displays its zenith during the dark phase and is caused by the diurnal rhythm in feeding, is not shared by pineal tryptophan which peaks near the end of the light phase (Sugden, 1979). There is, however, some disagreement over the presence of a pineal tryptophan rhythm since Mefford and Barchas (1980) and Young and Anderson (1982) have reported otherwise. Tryptophan loading in the rat induces a large increase in pineal 5-HT (Deguchi and Barchas, 1972; Young and Anderson, 1982) suggesting that the enzyme tryptophan hydroxylase is not fully saturated with this substrate. This effect appears to be species dependent since tryptophan loading has little influence on ovine pineal 5-HT levels (Sugden et al., 1985a). Sheep tryptophan hydroxylase is therefore saturated with respect to its substrate. Since these two mammals possess similar tryptophan concentrations and tryptophan hydroxylase activity, the implication is that either the two enzymes are structurally different, or the immediate environment in which they work is different to cause a lower K_m for the sheep enzyme. The activity of this enzyme is regulated by a beta$_1$-adrenergic receptor mechanism which utilizes the second messenger cyclic adenosine monophosphate (cAMP) (Shein and Wurtman, 1971). In the rat it has been reported to express a diurnal rhythm (Shibuya et al., 1978; Sitaram and Lees, 1978), attaining a two fold increase in night-time activity although this conflicts with Deguchi (1977) who reported an absence of

circadian change in the same animal. Conversely, Steinlechner et al. (1983) have demonstrated that peak activity for this enzyme in the Syrian hamster is reached during the daytime. The study by Chan and Ebadi (1980) emphasizes species variability since they could detect no circadian variation in tryptophan hydroxylase activity in the bovine pineal gland.

The activity of pineal 5-HTP decarboxylase is high as suggested by the low concentrations of pineal 5-HTP and by the administration of exogenous 5-HTP which increases pineal melatonin concentrations in rats (Wurzburger et al., 1976) and plasma melatonin levels in sheep (Namboodiri et al., 1983). King et al. (1984) have demonstrated a diurnal rhythm for endogenous rat pineal 5-HTP levels while Steinlechner et al. (1983) documented no circadian change in Syrian hamsters.

The pineal gland contains the highest concentration of 5-HT in the body (Saavedra et al., 1973) and it was thought to constitute an additional functional hormone of the gland since Garrick et al. (1983) described a pineal dependent circadian rhythm of CSF 5-HT levels. However, the indoleamine concerned has since been shown to be N-acetylserotonin (Taylor et al., 1985). Although a definitive function other than a precurser for melatonin synthesis has yet to be properly ascribed to 5-HT, a role in modulating adrenergic responses on pinealocytes via 5-HT receptors has been proposed (Sugden, 1989). Stimulation of pineal glands *in vitro* by noradrenaline (NA) has been shown to release tritiated 5-HT into the incubation medium (Aloyo and Walker, 1987; Aloyo and Walker, 1988). This efflux also occurs in denervated glands indicating that the 5-HT originates from pinealocytes, (rather than as a co-transmitter from presynaptic adrenergic nerve terminals), is dose-dependent and

inhibited by prazosin, an alpha$_1$-adrenergic antagonist (Aloyo and Walker, 1988). In addition, radioligand binding sites for 5-HT have been identified in the bovine pineal gland (Ebadi and Govitrapong, 1986) but have yet to be characterized. Using this evidence Sugden (1989) suggests that 5-HT may be involved in regulating the alpha$_1$-adrenergic potentiation of beta$_1$-adrenergic stimulation of cAMP and therefore melatonin synthesis. Alpha$_2$-adrenergic receptors are also thought to be involved, being located presynaptically to modify the release of NA (Lewy et al., 1986; Palazidou et al., 1989).

Light acts via the RHT to suppress NA release from the SCG-derived postganglionic terminals in the pineal. Darkness removes this inhibitory effect allowing NA to be released to stimulate beta$_1$-adrenergic receptors (Parfitt et al., 1976; Zatz et al., 1976) located on the pinealocyte membrane (Auerbach et al., 1981; Foldes et al., 1983; Craft et al., 1985). Stimulation of this receptor activates adenylate cyclase via a stimulatory guanine nucleotide binding regulatory protein, G_s, leading to the synthesis of cAMP and, in turn, the stimulation of SNAT (Klein and Berg, 1970). Rapid accumulation of cAMP, attaining maximum levels within 10 minutes following NA administration to cultured pinealocytes (Vanecek et al., 1985), mediates the induction of SNAT by activating a cAMP-dependent protein kinase. Progressive desensitization of the beta-adrenoceptor soon after the onset of darkness probably accounts for the gradual decline in cAMP levels after 10 minutes of NA stimulation (Vanecek et al. 1985). Desensitization is brought about by two mechanisms: the first is a circadian variation in the density of beta-adrenergic receptors located on the pinealocyte membrane which expresses a peak at the end of the light phase when

stimulation of the receptor has been minimal for some hours; the second is caused by a reduction in affinity of the beta-receptor for its endogenous ligand due to the activation of a beta-adrenergic protein kinase which phosphorylates the occupied adrenoceptor (Lefkowitz et al., 1986). An additional mechanism which would reduce the prolongation of the initial surge in pineal cAMP levels is an increase in cAMP phosphodiesterase activity induced as a result of a lag-period (Minneman and Iversen, 1976) which is in turn regulated by an interactive alpha- and beta-adrenergic mechanism (Vacas et al., 1985).

Beta-adrenergic stimulation of cAMP and SNAT is markedly enhanced by simultaneous activation of an alpha-adrenoceptor also located on the pinealocyte membrane (Klein et al., 1983b; Vanecek et al. 1985). Subsequent radioligand binding studies have characterized the receptors as being of the $alpha_1$-adrenergic subtype (Sugden and Klein, 1984; Sugden et al., 1985b). The increase of phosphatidylinositol (PI) turnover as a result of alpha-adrenergic receptor activation appears to involve protein kinase C (PKC), a Ca^{2+}-activated, phospholipid-dependent protein kinase as suggested by Sugden et al. (1985b) and Zatz (1985a). These workers measured cAMP levels and SNAT activity respectively and demonstrated that both could be increased by the administration of phorbol esters and the beta-adrenergic agonist isoprenaline. Phorbol esters in the absence of a beta-adrenergic agonist had no effect on cAMP and SNAT levels. Earlier in 1978, Zatz and Romero demonstrated that chelation of extracellular calcium inhibited the isoprenaline-induced or cAMP-induced induction of SNAT, indicating that an influx of calcium into the cytoplasm was required. This was later supported by studies on pineal explants and cultured pinealocytes indicate that inositol

monophosphate is the major product of PI hydrolysis. The negligible quantities of inositol biphosphate and triphosphate found by Ho et al. (1987a) and Zatz (1985b) suggest that an alternative means of mobilizing intracellular Ca^{2+} is utilized. An increase in intracellular Ca^{2+} induced by alpha-adrenergic receptor activation has been observed by Sugden et al. (1987). This increase, it has been proposed, is brought about by the opening of a ligand-dependent channel rather than a voltage-dependent channel since inhibition by nifedipine is not observed (Sugden et al., 1987). Activation of this channel probably involves PI-induced production of the endogenous activator of PKC, diacylglycerol, which encourages PKC to be redistributed from the cytoplasm to its "active" site - the cell membrane - and subsequently becomes responsible for amplifying the beta-adrenergic signal (Sugden et al., 1985c). Indirect evidence in support of this is given by Sugden and Klein (1988), who have shown that the amplified beta-adrenergic response induced by alpha-adrenergic agonists (e.g. phenylephrine) or phorbol esters is unaltered in pinealocytes pretreated with phosphodiesterase inhibitors which eliminates inhibition of cAMP metabolism by inhibited phosphodiesterase as a mechanism of potentiation. They suggest that phosphorylation of G_s or adenylate cyclase by the activated PKC as the most plausible explanation.

In addition to the above mentioned negative feedback mechanism of beta-adrenergic receptors, Sugden et al. (1988) have identified evidence indicating an equivalent action involving the desensitization of alpha-adrenergic receptors. This is probably brought about by the phosphorylation of the receptor by activated PKC (Leeb-Lundberg et al., 1986). The physiological implications of this were spelled out by Sugden (1989). The onset of night, allowing

the release of NA, leads to a massive surge of cAMP levels which may well be required to support the transcription of mRNA. Elevated mRNA levels accompanying the increase in SNAT activity are thought to be required for manufacturing either more SNAT molecules or an activator protein for the enzyme (Morrissey and Lovenberg, 1978a; Morrissey and Lovenberg, 1978b; Romero et al., 1975).

The role of cyclic guanine monophosphate (cGMP) in pinealocytes has yet to be elucidated. Its mention is warranted on the basis of a similarity with cAMP in the mechansims by which cGMP is regulated. As with cAMP, NA greatly elevates cGMP levels (O'Dea and Zatz, 1976; Vanecek et al., 1985) by an alpha- and beta-adrenergic receptor interaction (Vanecek et al., 1985; Vanecek et al., 1986). Alone, beta-adrenergic stimulation approximately doubles cGMP levels, unlike alpha-adrenergic stimulation alone which has no effect. However, if activated together, cGMP levels are elevated 100-fold (Vanecek et al., 1985). There appears to be an inverse relationship between the two second messenger cascade components that has been dubbed "see-saw signal processing" by Klein et al. (1981). This group discovered that inducing sympathetic denervation, either by constant light (LL) conditions or by superior cervical ganglionectomy elicited opposing effects for the pinealocyte cAMP and cGMP systems - the cAMP response becomes super-senstitive, whereas the cGMP response is diminished.

Unlike the well-defined diurnal variation in activity displayed by SNAT, a circadian rhythm in HIOMT activity as first reported by Axelrod et al. in 1965, is still in dispute (Ebadi, 1984). Its activity appears to be regulated by an adrenergic mechanism since Sugden et al. (1983a, 1983b, 1983c) have illustrated that LL or pineal sympathectomy-induced reduction in HIOMT activity

is reversed by beta-adrenergic agonists, e.g. isoprenaline or NA. The regular nocturnal stimulation of the pineal gland by NA is thought to be necessary to maintain HIOMT levels. In constant dark (DD) the opposite occurs, HIOMT levels become and remain elevated in contrast to SNAT levels which express a freerunning circadian rhythm. Changes in HIOMT activity have been identified but are expressed slowly over a period of days (Moore and Rapport, 1971; Klein and Moore, 1979). This fact, together with the results of Eichler and Moore (1971) which show a correlation between high HIOMT levels and testicular weight in hamsters, has prompted Binkley (1988) to suggest that HIOMT could regulate the amplitude of the melatonin produced on a seasonal basis, while SNAT is responsible for the production of the daily cycle.

The picture of melatonin synthesis is further complicated by the role of other components in pineal biochemistry, for example, vasoactive intestinal peptide (VIP) and prostaglandins. Direct evidence for the involvement of VIP in the physiological regulation of melatonin is presently lacking but since its first identification in the pineal by Uddman et al. (1980) VIP receptors have been characterized on the pinealocyte (Kaku et al., 1983). Furthermore, VIP has been shown to induce SNAT activity (Kaneko et al., 1980; Yuwiler, 1983a, 1983b) and increase the levels of both cAMP (Kaneko et al., 1980) and cGMP (Ho et al., 1987b). Recently, Chik et al. (1988a, 1988b) have demonstrated the involvement of alpha$_1$-adrenergic receptors in potentiating the VIP-induced stimulation of cAMP and cGMP through the probable use of PKC. The involvement of prostaglandins in modulating melatonin synthesis may well be a side effect of alpha$_1$-adrenergic stimulation which activates phospholipase A$_2$. This has been shown to cause an efflux of

arachidonic acid from the pinealocyte (Ho and Klein, 1987) which may be the source of PGE_2 Cardinali et al. (1982), using pineal explants, demonstrated as inducing an increase in the melatonin content of the medium.

1.1.6. Physiology of Melatonin

There are many factors involved in the control of pineal melatonin synthesis at both the extracellular and intracellular level. Environmental lighting plays a pivotal role in the external control of melatonin production (Wurtman et al., 1963). Melatonin is a nocturnal hormone, the mechanism by which it is synthesized being shut-off during the hours of day-light. In all species of animal so far investigated which produce melatonin, whether diurnally or nocturnally active its peak production occurs during the first half of the scotoperiod (dark period). The actual plasma levels of melatonin present an approximately symmetrical picture during the dark phase (Wilkinson et al., 1977; Waldhauser al., 1984). However, the duration of melatonin synthesis varies according to the length of the photoperiod. This has since been implicated as an important regulatory factor in photoperiodism - the ability to exploit the number of light hours in a day to modulate a seasonal activity, the best example being seasonal breeding animals.

Early studies identified a factor in the pineal gland that prevented gonadal maturation in rodents (Kitay and Altschule, 1954). Prepubertal pinealectomy increased ovarian and testes weight (Simonnet et al., 1951; Wurtman et al., 1959), an effect subsequently shown to be reversed by the administration of bovine pineal extract (Wurtman et al., 1959). However, identifying the active principle(s) involved proved slow due to the inconsistent

responses produced by melatonin in rodents (Chu et al., 1964). Additionally, contradictory results were obtained when various workers tested the reproductive responses to constant release melatonin capsules in certain rodent species (Reiter et al., 1974; Turek et al., 1976; Lynch et al., 1978;). It has since been shown to be a physiological action mediated by light (see Stetson and Watson-Whitmyre, 1984, and references cited therein). Plasma melatonin levels are higher during the short days of winter than in the summer which would appear to be due to the lengthening scotoperiod - as the duration of the dark period increases, so does the duration of melatonin production. This has a far-reaching effect in terms of reproduction. The golden hamster, for example, is a "long-day breeder"; a day-length greater than the "critical photoperiod" of 12.5 hours is required to maintain gonadal activity, whereas shorter day-lengths promote gonadal regression (Gaston and Menaker, 1967; Elliott et al., 1972; Elliott, 1976). Pinealectomy prevents these inhibitory responses to a short day-length whereas exogenous melatonin duplicates the effect of gonadal regression if administered during the "late afternoon" of the photoperiod or at the time approximating to "lights on". This, it was noted, artificially extends the duration of melatonin in the blood (Tamarkin et al., 1976). Melatonin administered at other times proved ineffective suggesting the existence of a diurnal rhythm in gonad sensitivity. Subcutaneous implants that produce a continuous release of melatonin were found to induce testicular regression in Syrian hamsters maintained under a long-daylength schedule (Turek et al. 1975). Interestingly, pinealectomized hamsters that receive a single injection dose of melatonin do not experience gonadal regression. For melatonin to be effective it must be given as an

infusion or as several bolus injections over an appropriate period that can be in the light or the dark phase (Carter and Goldman, 1983a; Bittman, 1984). This antigonadotropic action of melatonin is well established. Equally established, but somewhat contradictory, is the ability of melatonin to inhibit gonadal regression in certain circumstances - a property termed by Reiter (1980) as being "counterantigonadotropic". He found that subcutaneous melatonin implants prevented the gonadal regression in male Syrian hamsters transferred from long to short day-length conditions (Reiter et al., 1974). In addition melatonin implants inhibit testicular regression induced by normally effective evening injections of melatonin (Reiter et al., 1977). Carter and Goldman (1983b) provided evidence for a more active "progonadal" effect of melatonin with with the report that precisely timed daily infusions of this hormone elicited gonadal development in pinealectomized juvenile male Djungarian hamsters which was partially the result of melatonin exhibiting a stimulatory action on the secretion of follicle-stimulating hormone (FSH) and prolactin (PRL). The explanation considered most likely for the properties of melatonin is that it mediates its effects via an interaction with a specific receptor that undergoes a circadian rhythm in sensitivity.

1.1.7. Melatonin Receptors

Early studies using standard ligand binding techniques utilized [^{3}H]-melatonin and located various potential sites on both peripheral and central structures (Cohen et al., 1978; Niles et al., 1979). These reports were inconsistent mainly due to the specific activity of [^{3}H]-melatonin. The advent of *in vitro* autoradiography and the development of the radioligand 2-[^{125}I]-iodomelatonin has

considerably improved the prospects for future melatonin receptor research and already provided significant insights into its loci of action. Early studies demonstrated that many structures of the brain were capable of concentrating [^{3}H]-melatonin (e.g. Anton-Tay and Wurtman, 1969) which were taken as potential sites of action for endogenous melatonin. Evidence gradually accumulated to implicate the hypothalamus as the most likely candidate. It readily took up [^{3}H]-melatonin (Anton-Tay and Wurtman, 1969) and constant release melatonin implants located in the SCN region of the hypothalamus inhibited the reproductive activity of white-footed mice (Glass and Lynch, 1981). Melatonin had been shown to exert a significant effect on the protein microtubule content of the hypothalamus (Cardinali and Friere, 1975; Cardinali et al., 1975). Demaine (1979) demonstrated that melatonin modified the rate of discharge of spontaneously active hypothalamic neurones. Interestingly, Demaine (1979) also reported the ability of melatonin to reduce or abolish the inhibition of activity caused by dopamine. In addition, the presence of both membrane and cytosolic binding sites had been reported in the hypothalamus (Cardinali et al., 1979; Niles et al., 1979). It was realized though that the hypothalamus was not likely to be the only site of action. Cohen et al. (1978) had reported peripheral cytoplasmic binding sites for [^{3}H]-melatonin most concentrated in the ovary and uterus. Recently high affinity melatonin binding sites have been located on membranes prepared from the pars tuberalis of the adenohypophysis (Vanecek et al, 1987; Williams and Morgan, 1988; Morgan et al., 1989) of both rat and sheep using 2-[^{125}I]-iodomelatonin as the radioligand. This binding site was subsequently linked to a G-protein due to the ability of guanine triphosphate (GTP) to regulate its affinity with melatonin

(Morgan et al., 1989). The linking of a binding site to a "classical" second messenger system gives more credence to its claim as a receptor. In addition, a receptor at this site is consistent with the effects of melatonin on reproduction and its mediation via the hypothalamic-pituitary axis (Glass and Lynch, 1981). The work of Dubocovich has identified the retina as a further site of action for melatonin in addition to providing more information on the interaction between melatonin and dopamine. Picomolar concentrations of melatonin inhibit the calcium dependent release of [^{3}H]-dopamine from the rabbit retina (Dubocovich, 1983). It was subsequently shown that the efficacy of melatonin analogues in eliciting this effect depended upon the identity of the chemical group located on position 5 of the indole nucleus, but affinity (and therefore antagonist potential) was determined by the moiety substituted on position 3 (Dubocovich et al., 1985). It has since been established that this functional property of melatonin is associated with a binding site, determined using 2-[^{125}I]-iodomelatonin (Dubocovich and Takahashi, 1987).

Zisapel et al. (1988), having identified melatonin binding sites in the striatum and the hypothalamus reported a circadian rhythm of binding site density (but not affinity) only in the hypothalamus. Interestingly, the zenith of this rhythm would appear to be towards the end of the photophase. This does not concur with the report by the same group that the circadian rhythm of the inhibition of dopamine release from the hypothalamus by melatonin is approximately minimal at this time (Zisapel et al., 1985). Moreover, autoradiography has been used to provide evidence for a circadian rhythm in density of melatonin binding sites in the SCN region of

the rat hypothalamus (Laitinen et al., 1989) which also has its nadir at approximately the end of the dark phase. Autoradiography has revealed that potentially only a few central regions of the rat possess specific melatonin binding properties: the SCN, the median eminence; the choroid plexus and the anterior pituitary (Vanecek, 1988). It would appear that the distribution of these binding sites is much more widespread in the hamster (Weaver et al., 1987, 1988, 1988b; Vanecek and Jansky, 1989 from Morgan and Williams, 1989). Differing orders of potency and dissimilar K_i values between binding sites in the chicken retina and the hamster brain have led Dubocovich (1988) to propose the existence of two melatonin receptors. ML1 possesses high affinity for melatonin and is found in the chicken retina and brain, ML2 is a low affinity site located in the hamster brain (Dubocovich, 1988; Morgan and Williams, 1989).

Inherent in the term "melatonin receptor" is the concept of structural analogues that might act as antagonists at these sites. Early studies identified N-acetyltryptamine as being capable of inhibiting the blanching effect of melatonin on frog skin and was without activity itself (Heward and Hadley, 1975). 2-benzyl-N-acetyltryptamine (luzindole) was developed from N-acetyltryptamine and has a similar antagonistic profile (Dubocovich, 1985) though whereas N-acetyltryptamine appears to be a partial agonist with respect to the inhibitory effect of melatonin on dopamine release in the rabbit retina, luzindole elicits full competitive antagonism in both the chicken and the rabbit retina (Dubocovich, 1988). Despite the absence of an N-acetyl group (considered important for binding) N-(2,4-dinitrophenyl)-5-methoxytryptamine (ML23) has been reported to possess antagonistic activity (Laudon et al., 1988; Zisapel, 1988). In the rat it blocks both delayed maturation and inhibition

of ovulation induced by melatonin in addition to the inhibitory effect of melatonin on dopamine release in the hypothalamus (Laudon et al., 1988; Zisapel and Laudon, 1987; Zisapel, 1988). However, this ability is not reproduced in the rabbit retina and ML23 also fails to inhibit the reproductive effects of melatonin in the Soay ram (Dubocovich, 1988; Lincoln and Kelly, 1988).

1.1.8. Pharmacology of Melatonin

The first pharmacological (and physiological) action attributed to melatonin was a blanching effect it exerted on tadpoles (McCord and Allen, 1917; Lerner et al., 1958). Its potency was great enough for the technique to be adopted as a bioassay for many years until the more quantitative radioimmunoassay test was developed. Melatonin is essentially inert in numerous pharmacological tests except sleep where it has been shown to extend barbiturate-induced sleeping time (Barchas et al., 1967; Holmes and Sugden, 1982). Several studies have confirmed a hypnotic action though the underlying mechanism is not understood. It is conceivable that inhibited metabolism of the barbiturate accounts for the prolonged barbiturate-induced sleeping time since they share the same metabolic enzymes in the liver (Wurtman et al., 1968; Datta and King, 1980). However, intranasal, intravenous or oral administration of melatonin have been reported as effective at inducing sleep or reducing the time to sleep-onset in humans (Vollrath et al., 1981; Cramer et al., 1974; Anton-Tay et al., 1971) and hypothalamic implants of crystalline melatonin in cats also induce sleep (Marzcynski et al., 1964). Conversely, it should be mentioned that Mendelson et al. (1980) claimed that melatonin induced arousal in the rat at much lower doses than those required

to elicit hypnosis, while Chamblin (1973) failed to find a significant effect on any stage in sleep in cats. More recent studies of melatonin and sleep have implicated the serotonergic system, specifically the 5-HT_2 receptor, as a target for a melatonin-mediated modulating effect (Dugovic et al., 1988; 1989a; 1989b). Additionally, Waldhauser et al. (1990) proposed that the sleep-inducing properties of melatonin are the result of accelerating the initiation of sleep and improving its maintenance.

Sugden (1980) endeavoured to establish the toxicology of melatonin and demonstrated that large doses (i.e. >100mg/kg) were required to impair performance in a variety of tests (e.g. righting reflex, rotor-rod test). Animals recovered with no apparent ill-effects. Regardless of the route of administration melatonin was shown to possess negligible toxicity (LD_{50} >1000mg/kg using i.p., s.c. and oral routes of administration). Melatonin has also been shown to affect mood and performance in humans (Leiberman and Lea, 1988, and references cited therein). An early study by Kovacs et al. (1974) investigated low doses of melatonin on certain conditioned behaviours and found that a daily dose of 50ug/rat facilitated the extinction of active avoidance reflex and decreased the intertrial activity during extinction. It was also noted that melatonin facilitated passive avoidance behaviour using the electrified drinking spout in water-deprived rats paradigm. In a review by Datta and King (1980) it is suggested that melatonin might elicit these effects by influencing the "memory fixation process" but there is little hard evidence to support this proposal.

The action of melatonin on locomotor activity has been subject to a number of studies with conflicting results (Kovacs et al., 1974; Wong and Whiteside, 1968; Kastin et al., 1973; Sugden,

1980). The work described in chapter 3 also examined the relationship between melatonin and locomotor activity though only in the context of 5-HT-induced abnormal activity. Its action on the circadian rhythm of locomotor activity has received more favourable attention and is reviewed in greater detail in chapter 5. Briefly, Redman et al. (1983) demonstrated that a free-running locomotor activity rhythm in rats could be entrained by appropriately timed s.c. injections of melatonin. Further work by the same group has established a "dose-response curve" and the importance of an intact SCN for the action to be observed (Cassone et al., 1986a; Cassone et al., 1986b). Cassone et al. (1986a) were able to show that as little as 50ug/kg of melatonin produced entrainment. Together with the work of Kovacs et al. (1974) mentioned above and the early bioassay technique, this demonstrates the potency of melatonin in eliciting a pharmacological effect in an appropriate model.

1.2. Circadian Rhythms

1.2.1. Background

Until comparatively recently in the history of science rhythmic fluctuations in the day to day management of the life of an organism was taken for granted by the scientific community. It was assumed that the environmental light signal of dawn induced activity or rest according to the ecological niche occupied by the organism. The first doubts on this passive acceptance began in 1729 when the Frenchman de Mairan observed that the daily leaf movements exhibited by the Mimosa plant persisted in constant darkness (DD). De Marian concluded, wrongly, that an environmental factor other than light or dark was involved in the regulation of rhythms. Systematic investigation of this phenomenon was eventually begun this century

with Richter's report on rodent activity in constant conditions (Richter, 1922). This was followed by the work of Johnson (1926) who, as a result of investigating wild mice (*Peromyscus leucopus*), first postulated the existence of a "self-winding and self-regulatory clock" (Johnson, 1939). The concept of an endogenous clock did not gain wide acceptance, however, until the 1950's (Pittendrigh, 1954).

Biological rhythms are now recognised in almost every eukaryotic organism and range from a few milliseconds in period length (e.g. electrical activity of the brain) to several years. The origin of geophysical rhythms is thought to have derived from early life forms that had to cope with extreme variations in for example, damaging UV light exposure as the earth rotated on its axis approximately every 24 h. It became an advantage to be able to predict the onset of light and dark in order for the organism to restrict its UV sensitive activities to the dark phase. The term circadian (circa="about", diem="day") was introduced by Halberg (1959) to describe those rhythmic fluctuations that, when subjected to a constant environment would free-run with a period approximating to 24 h. The prefix "circa" has since been added to tidal, lunar and annual to describe other geophysically-derived rhythms which express periods of approximately 12 h, 28 days and 365 days respectively. The terms infradian and ultradian have been introduced to distinguish between those rhythms that are greater or shorter than 24 h respectively. This section will concentrate on the physiology and mechanisms of circadian rhythms.

The fundamental characteristic of a proposed circadian process, which can be physiological, behavioural or biochemical in nature, is that it free-runs, i.e. in a constant environment it

expresses a period approximating to but not exactly equal to 24 h and can be synchronized (or entrained) by the appropriate time-cue (or "zeitgeber" - literally "time-giver"). In the entrained state, therefore, there is a constant phase angle difference between the rhythm and the zeitgeber (Aschoff et al., 1982). It is apparent that the circadian clock controlling the rhythm of locomotor activity expressed by, for example, a nocturnal rodent, must be continually reset by the environmental light/dark cycle - the most important natural zeitgeber. This is achieved by a daily phase-shift in the onset of locomotor activity. Thus a mechanism must exist which allows the organism to "read" the appropriate incoming environmental signals and adjust its behaviour accordingly. Initial investigations examined the role of the visual system in the entrainment mechanism since blinding elicited free-running rhythms in the absence of zeitgebers other than the standard light-dark (LD) cycle (Richter, 1965). However, earlier work by Altman (1962) had shown that lesioning the visual cortex or superior colliculus, failed to disrupt the diurnal rhythm of locomotor activity. In addition, Chase et al. (1969), Moore and Eichler (1972) and Stephan and Zucker (1972) demonstrated that lesioning of the primary and accessory optic tracts also failed to abolish entrainment. Reason dictated that there were two components to the light information transmitted from the retina that only became distinguishable after the optic chiasm. Earlier work by Richter (1967) established the importance of the hypothalamus in regulating circadian rhythms by demonstrating that lesions of the ventral median region of the hypothalamus disrupted circadian locomotor activity. As a result the retino-hypothalamic tract (RHT) itself was finally identified in mammals by two independent autoradiography studies of the visual

system (Hendrikson et al., 1972; Moore and Lenn, 1972). As briefly described above in the section on melatonin, this tract, originating from the retina, terminates in the SCN of the hypothalamus (Moore, 1973). Unlike blinded rats which express free-running rhythms as if in DD, the destruction of this area was found to induce arrhythmicity in the circadian rhythms of adrenal corticosterone content (Moore and Eichler, 1972), locomotor activity and drinking behaviour (Stephan and Zucker, 1972).

1.2.2. Neural Regulation of Circadian Rhythms

Visual stimuli from ganglion cells are transmitted to the optic chiasm bilaterally along the optic nerves. The largest of the four projections leaving the chiasm, the primary optic tract (POT), has components that terminate in the superior colliculus, pretectal nuclei and lateral geniculate nucleus (cited from Rusak and Zucker, 1979). The superior and inferior accessory optic tracts (SAOT and IAOT, respectively) originating from the chiasm terminate in the midbrain tegmentum. Lesions of these pathways have been shown to elicit abnormal free-running rhythms, for example Rusak (1977) reports elongated periods or an extension in the active phase or a reduced rate of re-entrainment to a 12 h phase shift in the light-dark cycle. It should be mentioned that such lesions are difficult to restrict to the tracts themselves and Rusak admits damage to adjacent structures that offer input to the SCN. Thus the involvement of the primary and accessory optic tracts in the mechanism of entrainment remains tentative and unproven. The fourth tract, the RHT, carries photic information directly to the SCN, preferentially terminating in the ventral and caudal regions of the nuclei and forming both Gray-type I and Gray-type II synapses

suggesting both excitatory and inhibitory components (Rusak and Zucker, 1979). The SCN is a bilateral structure that resides immediately superior to the optic chiasm and ventrolateral to the third ventricle. It projects to and receives input from: the anterior hypothalamic area, the paraventricular nucleus, the retrochiasmatic area, several nuclei of the tuberal hypothalamus, the paraventricular thalamic nucleus, the midbrain periaqueductal gray, and the contralateral SCN. It receives input only from ascending serotonergic fibres of the midbrain raphe nuclei, and the ventral lateral geniculate nucleus in addition to the retina. Structures that receive output only from the SCN are the lateral hypothalamic area and the lateral septal nucleus (Rusak and Zucker, 1979; Moore, 1983). There are no long ascending or descending projections from the SCN suggesting that processing of its output is performed at the hypothalamic level.

The SCN is now regarded as a major pacemaker for circadian rhythms in the body, but not necessarily the only one. As mentioned above SCN ablation results in arrthymicity in a number of rhythms provided at least 75% of the SCN has been destroyed (Moore, 1982). Circadian function is also eliminated by lesions in the rat neonate SCN and fails to recover during maturation to adulthood (Mosko and Moore, 1977). Schwartz et al. (1977, 1980) measured glucose consumption of the SCN using (^{14}C)-deoxyglucose and demonstrated that, unlike other brain structures, it expressed a circadian rhythm. This measure of metabolic activity was found to be high during the light phase and low during the dark phase - a result consistent with the electrophysiological studies of Inouye and Kawamura (1979). They detected high multiunit activity in the SCN during the day which became low at night, an effect resistant to

surgical isolation of the SCN. In addition, Rusak and Groos (1982) found that electrical stimulation of the nuclei affected both the tonic and phasic expression of free-running circadian rhythms in the rat and hamster. These results reflect the importance of the SCN as an oscillator for the circadian system but further evidence supports the existence of other oscillatory centres coupled together under entrained conditions. Stephan (1984) has been able to show that activity rhythms of SCN lesioned rats in DD entrained to a restricted food availability. Food available *ad libitum* induced arrythmia. The animals even displayed food anticipatory behaviour with an increase of activity prior to the provision of food, implying a memory pacemaker for feeding times. Clarke and Coleman (1986) have found that wheel-runnning and drinking rhythms persist in SCN-lesioned rats if food availability is alternated between schedules of restriction and *ad libitum*. It has been suggested that this reflects internal desynchronization of circadian rhythms after SCN lesioning rather than arrhythmicity (Mitchell, 1989). The locations of secondary oscillators are likely to reside in the near vicinity of the SCN due to its lack of long projections although a humoral input using perhaps melatonin has not yet been eliminated. Moore (1982, 1983) has suggested the most likely candidates are the lateral geniculate nuclei (LGN) retrochiasmatic area (RCN), the lateral hypothalamus (LH) and the ventral median hypothalamus (VMH).

Lesions of certain hypothalamic structures surrounding the SCN have yielded information concerning the regulation and generation of circadian rhythms though currently their value is limited. Damage to the VMH can reverse the normal pattern of feeding where a rat fed *ad libitum* ingests mainly during darkness (Kakolewski et al., 1971; Zucker, 1971). Alternatively such damage

may equalize the quantities of food ingested in the light and dark phases (Bernadis, 1973). The nocturnal drinking rhythm in rats is also attenuated by VMH lesions. In contrast, Rowland (1976) demonstrated that lesions to the LH increased the dark-phase appropriate behaviours of feeding and drinking. It is interesting to note that the LH has been shown to receive retinal input (Riley et al., 1980). These results are less surprising when one considers the importance of this region to the regulation of food intake. They may well be explained more by the hyperinsulinaemia and peripheral metabolic changes induced by VMH damage than by circadian rhythms (LeMagnen et al., 1973; Friedman and Stricker, 1976; Rusak and Zucker, 1979).

The influence of the ventral lateral geniculate nucleus (vLGN) has recently aroused more intense investigation. This receives a direct photic input from the retina via the retinolateral hypothalamic tract (RLHT). It therefore provides an indirect photic pathway from the retina to the SCN via the geniculo-hypothalamic tract (GHT) which originates from the region known as the intergeniculate leaflet (IGL, Swanson et al., 1974; Pickard, 1982, 1985; Card and Moore, 1982) and terminates in the ventral portion of the SCN (Rusak and Zucker, 1979). Harrington and Rusak (1986) have been able to show that IGL lesioned hamsters show reduced phase-advances in running wheel activity rhythms if subjected to an appropriately timed light pulse. No effect on phase-delays was observed. In agreement with this result Pickard et al. (1987) have also demonstrated that lesions of the IGL modify the amplitude of light pulse induced phase-shifts on free-running locomotor activity rhythms, though phase-delays were increased. In addition this study reported that such lesions lengthened the free-running period in DD

in comparison to non-lesioned control animals and reduced the normal lengthening of the period of animals maintained in constant light (LL) - a property that was dependent on the intensity of the incident light. From this Pickard et al. (1987) reasoned that the LGN partially mediates the tonic action of environmental lighting (i.e. determination of period length). The ability of an animal to remain entrained, with respect to wheel running activity rhythms, to a given LD cycle is not impaired following lesions to the IGL (Dark and Asdourian, 1975; Pickard et al. 1987) which suggests that it transmits information on photic signals regarding the intensity of environmental lighting as opposed to absolute illumination. However, following a 12 h phase shift in the LD cycle the activity rhythms of LGN-lesioned hamsters reentrain significantly more slowly than neurologically intact animals (Dark and Asdourian, 1975; Rusak, 1977). Recently Rusak et al. (1988) reported that in hamsters housed under LL or DD conditions electrical stimulation of the GHT induces mostly phase advances of wheel-running activity rhythms if given during the late subjective day or small phase delays if given during the late subjective night or early subjective day. It appears that GHT stimulation mimicks the effects of dark pulses (Rusak et al., 1988) in LL. The GHT is characterized in part by immuno-reactivity to neuropeptide Y (NPY) in its cells of origins and constitutes the major source of this peptide found in the SCN (Card and Moore, 1982; Harrington et al., 1985). It is thought that electrical stimulation of the LGN leads to the release of this peptide since its application directly into the SCN results in time-dependent phase-shifts in the free-running activity rhythms of hamsters (Albers and Ferris, 1984) which also mimic dark pulses in LL.

The precise role of the raphe nuclei (RN) in the regulation of circadian rhythms is still a matter of debate. Ascending serotonergic projections from the median and dorsal RN innervate the ventral region of the SCN (Rusak and Zucker, 1979) and the LGN (Azmitia and Segal, 1978; Moore et al., 1978). There is also evidence that the nuclei provide another indirect photic pathway to the SCN, since Foote et al. (1978) identified a retinal projection to the RN. Studies using both lesions of the SCN and serotonin depleting drugs have shown that the integrity of the RN is not required for entrainment of the locomotor activity rhythm to a LD schedule (Kam and Moberg, 1977; Honma et al., 1979; Levine et al., 1986). However, a lesioned animal subjected to a constant environment either DD or LL displays severe perturbation of the rhythm (Levine et al., 1986) while animals treated with *para*-chlorophenylalanine (pCPA) become arrhythmic for several days (Honma et al., 1979). Presumably this reflects the duration of 5-HT deprivation induced by the drug, although neither drugs nor lesioning removes all 5-HT. Electrical stimulation of the raphe reduces SCN activity (Bloom et al., 1972) as does direct application of 5-HT into the SCN (Meijer and Groos, 1988). The lack of responsiveness of SCN visual cells (i.e. those that respond to visual stimulation of the retina) to iontophoretic application of 5-HT agrees with the concensus that the median and dorsal RN exert a modulatory role on circadian rhythm expression rather than an absolute one. Interestingly, two recent reports have presented evidence that suggest serotonergic innervation of the SCN may be necessary for the ontogenic development of certain circadian rhythms (Banky et al., 1986, 1988).

1.3. 5-Hydroxytryptamine (5-HT)

1.3.1. Background

The majority of 5-HT, also known as serotonin, found in the body is located in the enterochromaffin cells of the gut and blood platelets. Only in the CNS which holds approximately 1-2% does it act as a neurotransmitter (Kruk and Pycock, 1983). Its isolation from the blood and the subsequent deduction of its structure was reported by Rapport (Rapport et al., 1948; Rapport, 1949). The peripheral pharmacology of 5-HT has, however, assumed a secondary importance with respect to scientific investigation since it was first identified in the brain (Twarog and Page, 1953). Central 5-HT pathways were originally mapped in 1964 by Dahlstrom and Fuxe using the Falck histofluorescence technique. It was found that 5-HT neurons stretched to practically every region of the brain but the cell bodies were located primarily in well defined clusters of the raphe nuclei which lie in the mid-portion of the pons and upper brain stem (Dahlstrom and Fuxe, 1964; Fuxe, 1965; Anden et al., 1966). Dahlstrom and Fuxe devised an alphanumeric-based nomenclature for the ascending and descending 5-HT pathways originating from this region. The more caudal 5-HT cells (B1 to B3) descend to the medulla, pons and spinal cord while the rostral ones (B5, B7 to B9) project to the diencephalon and forebrain. There are three ascending pathways projecting dorsally to the neostriatum, medially primarily to the substantia nigra and ventrally to innervate the diencephalon, limbic system and cortex. This wide distribution is consistent with a wide sphere of influence in central physiology for 5-HT. As a central neurotransmitter it has been implicated in playing a modulatory role in: sleep, thermoregulation, appetite, pain perception, control of pituitary secretions, sexual behaviour,

pituitary secretions, sexual behaviour, circadian rhythms, in the pathophysiology of migraine, depressive illness, anxiety, psychosis, and in the action of hallucinogenic drugs (Barchas and Usdin, 1973; Iversen, 1984; Roberts, 1984).

1.3.2. Synthesis and Metabolism

The essential steps of 5-HT synthesis have been outlined in section 1.1.4. and can be seen in Fig 1. It should be mentioned that central 5-HT is derived exclusively from the brain as the molecule is too polar to traverse the blood brain-barrier (BBB). Thus to increase endogenous brain levels of 5-HT, the immediate presursor in its biosynthesis, L-5HTP, is administered in combination with a decarboxylase inhibitor, e.g. carbidopa to prevent peripheral metabolism to 5-HT. Carbidopa fails to penetrate the brain which L-5HTP can enter freely and so be converted to 5-HT. Central 5-HT is thought to be derived from 5-HT neurons although recent evidence suggests that cerebrovascular endothelial cells might be capable of 5-HT synthesis (Maruki et al., 1984). The rate limiting step in neuronal 5-HT synthesis is the conversion of L-tryptophan to L-5HTP by tryptophan hydroxylase (Ashcroft et al., 1965). The location of this enzyme is restricted to only those cells capable of producing 5-HT. Despite this it would appear that tryptophan hydroxylase works *in vivo* at only 10-25% its capacity in comparison to ideal conditions *in vitro* (Carlsson, 1974; Gal and Whitacre, 1981). This would suggest along with data produced by Knowles and Pogson (1984) that *in vivo* the enzyme is not fully saturated hence the ability of the amino acid precursor of 5-HT, L-tryptophan, to exert a strong influence on 5-HT synthesis. Entry of tryptophan into the brain depends on the ratio of tryptophan

concentration in plasma to the concentration of other large neutral amino acids that compete with tryptophan for same brain uptake mechanism (Fernstrom, 1981). This ratio can be altered by drugs or carbohydrate metabolism that lead to insulin secretion which increases brain uptake of tryptophan thereby increasing brain 5-HT levels.

Central 5-HT is deactivated pre- and postsynaptically by conversion to 5-hydroxyindoleacetaldehyde by monoamine oxidase (MAO) (EC 1.4.3.4, amine: oxygen oxidoreductase (deaminating)(flavin-containing)) which is then rapidly oxidized to 5-hydroxyindoleacetic acid (5-HIAA). 5-HT in the pineal gland and certain other central and peripheral sites undergoes an alternative metabolic pathway as explained above to manufacture melatonin. Small amounts of 5-hydroxytryptophol, reduced from the aldehyde, have been detected in the brain though this is not considered an important elimination route for 5-HT (Cheiftefz and Warsh, 1980). MAO is a flavoprotein that exists in two forms, A and B, as first reported by Johnston (1968) and resides on the outer surface of mitochondria (Youdim and Findberg, 1983). Their distinction depends on their susceptibility to inhibition by either clorgyline which preferentially inhibits MAO type A, or deprenyl which prefers type B. Immnunological localization studies have identified MAO-B in 5-HT cells and glial cells while MAO-A is located in high concentrations in catecholamine neurons. Both types of enzyme can metabolize 5-HT, although MAO-A favours 5-HT and the catecholamines as substrates while MAO-B favours phenylethylamine, benzylamine and tryptamine (Neff et al., 1974; Cooper et al., 1986; Fowler and Tipton, 1982; Youdim and Findberg, 1983). An early concept that both types of MAO were the same enzyme but with two active sites according to the substrate

(White and Wu, 1975; Boullin, 1978) can be discounted since Cawthon et al. (1981) isolated the different forms and determined their respective molecular weights (63000 for type A and 60000 for type B) and peptide pattern.

1.3.3. Multiple 5-HT Receptors

Gaddum and Picarelli (1957) were the first to demonstrate the existence of two pharmacologically distinct receptors for 5-HT. They were labelled 'D' and 'M' according to the mechanism behind the ability of 5-HT to contract smooth muscle in the guinea pig ileum. The 'D' receptor elicited contraction directly and could be blocked by phenoxybenzamine (dibenzylamine, hence the label 'D') or the hallucinogen D-lysergic acid diethylamide (D-LSD). The 'M' receptor effected contraction indirectly through the release of acetylcholine which could be inhibited by morphine or cocaine. This system received much criticism for many years and has never been particularly applicable to the CNS, but recently attempts have been made to incorporate it into the current classification of 5-HT receptor subtypes. Early functional studies using microiontophoresis identified the main effect of 5-HT on the activity of neurons to be a reduction in excitability but in a few brain regions 5-HT evoked a facilitatory action (Roberts and Straughan, 1967; Bloom et al., 1972). To satisfy this dichotomous action it was proposed that there were at least two central 5-HT receptors, one of which was responsible for excitatory effects and subject to inhibition by the classic 5-HT antagonists (methysergide, cyproheptadine, metergoline and cinanserin); and one which effected inhibitory actions of 5-HT and was resistant to drug-induced antagonism. A modulatory effect has also been ascribed to 5-HT by

Aghajanian (1981), who reported that the current of iontophoretically applied glutamate required to produce the activation of facial motoneurons is significantly reduced in the presence of 5-HT. Based on electrophysiological evidence Aghajanian (1981) proposed the existence of three 5-HT receptors. S_1, located postsynaptically, exerts a modulatory influence on excitatory amino acid neurons and is readily blocked by the classic 5-HT antagonists. The second type of receptor, S_2, at which LSD acts as an agonist, is an autoreceptor, and inhibits the activity of cell bodies in the raphe nuclei. The third type of 5-HT receptor, S_3, is postsynaptic and suppresses neuronal activity. The standard antagonists are ineffective and LSD acts only as a weak partial agonist.

1.3.4. Radioligand Binding Studies

Radioligand binding has had the most significant impact on 5-HT receptor nomenclature in recent years. Early studies utilized [^{3}H]-5-HT and [^{3}H]-LSD as ligands to characterize high affinity 5-HT binding sites in the brain. Marchbanks (1967) demonstrated the existence of a high affinity binding site in rat synaptosomes but the non-hallucinogenic L-LSD was found to displace 5-HT as effectively as D-LSD and nonindolic psychotomimetics did not affect 5-HT binding. Later, however, Farrow and Van Vunakis (1973) identified a specific high affinity site in the cerebral cortex of the rat using [^{3}H]-LSD, which could be displaced by high concentrations of other hallucinogens (e.g. psilocin and mescaline) but not by their congeners (e.g. L-LSD). Subsequent work by Bennett and Aghajanian (1974), with an improved technique and using rat brain homogenates, demonstrated that there was a high and a low affinity component to the binding of D-LSD. L-LSD competed with the

binding of D-LSD at the low affinity site indicating that only the high affinity site was stereospecific. This latter site was subject to regional variations, being found in cerebral cortex, striatum and the midbrain. Destruction of the cell bodies at the raphe nuclei had minimal effect on [^{3}H]-LSD binding suggesting that a postsynaptic location of the binding sites. Bennett and Snyder (1975) extended this work to show that the non-hallucinogen 2-bromo-LSD was as potent as LSD in displacing [^{3}H]-LSD from brain membrane binding sites. This, together with the finding by Lovell and Freedman (1976) that hallucinogenic compounds chemically distinct from LSD failed to inhibit [^{3}H]-LSD binding, disproved the original concept that there was a single population of receptors responsible for the hallucinogenic effects of certain drugs. Bennett and Snyder (1976) continued their investigation of putative 5-HT binding sites by comparing the specific binding of [^{3}H]-5-HT and [^{3}H]-LSD to rat brain membranes. They showed that D-LSD was equally effective in displacing both [^{3}H]-5-HT and [^{3}H]-LSD from their respective binding sites, but 5-HT displayed a much greater affinity for the site labelled with [^{3}H]-5-HT. Several analogues of 5-HT also shared this property. A number of known 5-HT antagonists (e.g. cyproheptadine, methiothepin) though expressed a preference for the [^{3}H]-LSD labelled site. This led Bennett and Snyder to propose a two-state model for the 5-HT receptor where [^{3}H]-5-HT binds to the agonist state. Due to its equal affinity for both sites it was thought that [^{3}H]-LSD labelled both the agonist and antagonist states.

The discovery by Leysen (1978) that spiperone, previously considered to be a pure dopaminergic ligand, labelled the antagonist serotonergic sites in the rat frontal cortex, a region known to be sparsely populated with respect to dopamine receptors (Leysen and

Laduron, 1977), proved a significant turning point in 5-HT receptor research. It was followed in 1979 by the work of Peroutka and Snyder that has essentially established the foundation for modern 5-HT receptor nomenclature. On the basis of the differing binding characteristics of [^{3}H]-5-HT and [^{3}H]-spiperone Peroutka and Snyder suggested that 5-HT receptors be referred to as 5-HT$_1$ and 5-HT$_2$. [^{3}H]-5-HT expresses high affinity (nM range) for the 5-HT$_1$ site but only micromolar affinity for the 5-HT$_2$ site. [^{3}H]-spiperone exhibits nanomolar affinity for the 5-HT$_2$ site but possesses a much lower affinity for the 5-HT$_1$ site. Radiolabelled D-LSD has equal affinity for both sites (the B*max* obtained with [^{3}H]-LSD was found to correspond to the sum of B*max* for [^{3}H]-spiperone and B*max* for [^{3}H]-5-HT binding). Consistent with [^{3}H]-LSD binding sites, structurally related compounds, e.g. methysergide and metergoline, were found to displace both [^{3}H]-5-HT and [^{3}H]-spiperone from 5-HT$_1$ and 5-HT$_2$ binding sites to the same extent. 5-HT$_1$ binding sites have since been found in the highest concentrations in the prefrontal cortex, hippocampus, tuberculum olfactorium, striatum, thalamus and raphe nuclei. 5-HT$_2$ binding sites densely populate the prefrontal cortex, tuberculum olfactorium, nucleus accumbens and striatum (Leysen, 1985). It has been noted that the distribution of 5-HT$_1$ and 5-HT$_2$ binding sites corresponds neither to the regional brain distribution of specific 5-HT uptake nor to the distribution of 5-HT content in brain areas (Leysen and Tollenaere, 1982; Leysen et al., 1983). This inconsistency between 5-HT innervation and 5-HT binding sites has been used as an argument against the physiological significance of a binding site (Barbaccia et al., 1983).

The 5-HT$_1$ site as labelled by [^{3}H]-5-HT was soon shown to be heterogenous by Pedigo et al. (1981). Displacement of [^{3}H]-5-HT

binding by spiperone revealed two sites, one exhibiting high affinity (K_d in the nM range) for spiperone and the other low affinity (K_d in the micromolar range) which were designated 5-HT_{1A} and 5-HT_{1B} respectively. However, further investigations by Schnellmann et al. (1984) into tissue and species differences revealed this to be a too simplistic explanation of 5-HT_1 receptor heterogeneity. Realization of the existence of 5-HT receptor subtypes and the implications for behavioural pathophysiology inherent in their characterization encouraged the synthesis of novel compounds aimed at achieving selective agonist or antagonist action at a particular site. This had the effect of accelerating 5-HT research. Of particular importance was the synthesis of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT) by Arvidsonn et al. in 1981 which proved to be a highly selective ligand for 5-HT_{1A} receptors. The consequence of this was twofold: first was the synthesis of a family of piperazine derivatives all expressing nanomolar affinity for 5-HT_{1A} receptor sites (e.g. ipsapirone, buspirone, gepirone); and second was the ability to redefine 5-HT_1 receptors as those that express a high affinity for 8-OHDPAT (5-HT_{1A}) and those that express low affinity for 8-OHDPAT (5-HT_{1B}). 1981 also saw the introduction of ketanserin, an agent reported to block responses mediated by 5-HT_2 receptor activation. Subsequent ligand-binding studies confirmed that it possessed high affinity for 5-HT_2 binding sites and negligible affinity for 5-HT_1 sites (Leysen et al., 1982). This was a major breakthrough since existing "classical" 5-HT antagonists had been plagued by lack of specificity, e.g. spiperone also expressed high affinity for dopamine-2 and 5-HT_{1A} receptor sites (Leysen et al., 1978). Use of [^{3}H]-mianserin had been troubled by its affinity for histaminergic-1

(H1) receptors. Although [^{3}H]-ketanserin possessed moderate affinity for H1 and alpha$_1$ adrenergic receptor sites provided low nanomolar concentrations were used these were not labelled (Leysen et al., 1981; Leysen et al., 1982).

Autoradiography provided the evidence for another 5-HT$_1$ receptor subtype identified in high concentrations in the choroid plexus of the rat and porcine brain, which was designated 5-HT$_{1C}$ (Cortes et al., 1984; Pazos et al., 1984). The 5-HT$_{1C}$ receptor possessed high affinity for 5-HT, as demanded by the criteria for a 5-HT$_1$ receptor (Peroutka and Snyder, 1981) and could be labelled by [^{3}H]-mesulergine and [^{3}H]-LSD, but putative selective ligands for 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_2$ receptors displayed low affinity. Although initially greeted with scepticism by 5-HT investigators, doubt has since been replaced by recognition that multiple 5-HT receptor subtypes are likely to prove the norm. This proved prophetic upon the identification of a fourth 5-HT$_1$ receptor subtype now known as 5-HT$_{1D}$. It was first reported by Heuring and Peroutka (1987) in bovine brain membranes and has since also been identified in pig and most importantly in man (Hoyer et al., 1988; Waeber et al., 1988). 5-HT$_{1D}$ receptors are characterized by displaying nanomolar affinity for [^{3}H]-5-HT and micromolar affinity for 5-HT$_{1A}$ selective ligands such as 8-OHDPAT, ipsapirone and buspirone. Agents that label 5-HT$_{1B}$ sites in rat brain (e.g. RU24969 and (-)-pindolol) are approximately two orders of magnitude less potent at 5-HT$_{1D}$ sites and the affinities of 5-HT$_2$ and 5-HT$_3$ selective ligands are negligible. To identify further 5-HT receptor subtypes research groups are now required to use cocktail-style incubation mediums containing the appropriate selective ligands in sufficiently high concentrations to inhibit the binding of the crucial ligand to non-target binding

sites. Using this procedure Sumner and Humphrey (1989) have reported that two subpopulations of 5-HT_{1D} binding sites can be discerned in porcine brain, one of which shares the binding characteristics of the novel 5-HT_1 receptor subtype, 5-HT_{1E}, identified and named by Leonhardt et al. (1989). It remains to be seen as to whether these sites can withstand peer scrutiny and be allocated a function.

Having had a quiet decade as the best functionally characterized 5-HT receptor, the 5-HT_2 receptor is now receiving its share of controversy. Previously thought of as a single entity, differing opinions on the precise heterogeneity of this receptor are being voiced. The classification of 5-HT receptors has always been hindered by the lack of suitably selective antagonist molecules for 5-HT_1 receptor/receptor subtypes and selective agonist molecules for the 5-HT_2 site. The hallucinogenic amphetamine derivative, 2,5-dimethoxy-4-methylamphetamine (DOM) and its halogen homologues 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (DOB) and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), had long been suspected of acting through the serotonergic system but it was not until the manufacture of the equivalent radioligands that their 5-HT_2 receptor binding properties was fully appreciated (e.g. Lyon et al., 1986). Titler and colleagues argue that these compounds label a high affinity "agonist" state of the 5-HT_2 receptor (Titler et al., 1987; Leonardt and Titler, 1989), while [^{3}H]-ketanserin displays equal affinity for this high affinity state and a low affinity "antagonist" state of the receptor (reminiscent of Bennett and Snyder's two state 5-HT receptor model of 1976). Conversely, Peroutka and colleagues interpret the evidence as indicating the existence of 5-HT_{2A} and 5-HT_{2B} (Wang et al., 1988; Pierce and Peroutka, 1989). The matter has yet to be resolved (see Strange,

1990 and Peroutka, 1990) despite the recent cloning of the 5-HT_2 receptor (Pritchett et al., 1988).

The presence of 5-HT_3 receptors in the CNS was not revealed until quite recently (Kilpatrick et al., 1987). Previously they had been characterized pharmacologically in the periphery by criteria described by Bradley et al. (1986). They categorized a response as being mediated by 5-HT_3 receptors if:

(a) It could be inhibited by the recognized selective 5-HT_3 antagonists (-)cocaine, MDL 72222 (3,5-dichlorobenzoyltropine ester) or ICS 205-930 (3*a*-tropanyl)-1H-indole-3-carboxylic acid ester).

(b) 5-HT_1 and 5-HT_2 antagonists were ineffective.

(c) The response was mimicked by 2-methyl-5-HT, a selective 5-HT_3 agonist of approximate equal potency to 5-HT.

The synthesis of selective ligands enabled central 5-HT_3 binding sites to be identified, which in turn revealed the affinity expressed by the endogenous ligand for the receptor which is variously reported as having a K_i of between 40 and 190nM (McKernan et al., 1990; Kilpatrick et al., 1987; Peroutka and Hamik, 1988) depending on the conditions used in the assay procedure. 5-HT_3 receptors are located in the highest concentrations in the cortex, hippocampus, amygdala, nucleus accumbens and tuberculum olfactorium (Kilpatrick et al., 1987; Milburn and Peroutka, 1989). Anomalies in the binding characterization of 5-HT_3 receptors between different species using the recognized agonists and antagonists have revealed potentially three subtypes, though attempts to designate them as 5-HT_{3A}, 5-HT_{3B} and 5-HT_{3C} have so far been resisted. A further example of the diverging and converging history of 5-HT receptor terminology is the attempt to revive the early 5-HT receptor classification described by Gaddum and Picarelli (1957). Bradley et

al. (1986) have proposed that the 5-HT$_3$ receptor is the modern-day equivalent of the 'M' receptor and the 5-HT$_2$ receptor corresponds to the 'D' receptor. This latter designation is generally accepted amongst the 5-HT research community but the suitability of the 5-HT$_3$/'M' linkage has been questioned (Fozard, 1987).

Soon after the recognition of 5-HT$_3$ receptors in the CNS (Kilpatrick et al., 1987) a further brief report was published also claiming the identification of a binding site in cultured mouse embryo colliculi neurons unsuited to 5-HT$_1$ or 5-HT$_2$ classification (Dumius et al., 1988) and positively coupled to adenylate cyclase. It was found that the 5-HT$_3$ ligand ICS 205-930 acted as a antagonist at this new site, albeit with much lower affinity, and proposed that it could be an embryonic form of the 5-HT$_3$ receptor. Subsequent analysis has revealed that this site was originally identified in guinea pig hippocampal membranes by Shenker et al. (1987). It bears little resemblence to any existing 5-HT receptor classes with regard to its biochemical pharmacology and has therefore been designated 5-HT$_4$ (Dumius et al., 1988).

1.3.5. Functional Correlates of 5-HT Receptors

Sceptics demanded evidence of functional relevance before assigning receptor status to binding sites (e.g. Leysen, 1984). The response was the publication of more detailed reports that correlated binding sites with behavioural data and/or biochemical data such that the site was linked with a second messenger system. The biochemical correlates will be considered first.

1.3.5.1. Biochemical Correlates

Peroutka and Snyder backed their original 5-HT receptor classification by the simultaneous publication that suggested a functional role for the 5-HT$_1$ receptor site (Peroutka and Snyder, 1979; Peroutka et al., 1979). They advanced that this receptor was regulated by guanidine nucleotides indicating a receptor-adenylate cyclase coupled system (Peroutka et al., 1979b). However, data reported by Nelson et al. (1980) showed that 5-HT stimulated adenylate cyclase and 5-HT$_1$ binding sites have differing regional and subcellular distributions. In addition, inhibition of radioligand binding by the presence of GTP or GDP is no longer considered synonomous with an adenylate cyclase effector system (Blackmore et al. 1985). There has since been published conflicting reports as to whether the 5-HT$_{1A}$ receptor is positively or negatively coupled to adenylate cyclase (Conn and Sanders-Bush, 1987; Barbaccia et al., 1983b; Shenker et al., 1985; Weiss et al., 1986; Markstein et al., 1986). De Vivo and Maayani (1985, 1986) have used forskolin, a pharmacological agent that bypasses the guanine nucleotide subunits to stimulate adenylate cyclase directly, to add to the evidence that activation of 5-HT$_{1A}$ receptors in rat and guinea pig hippocampal membranes inhibit cAMP production. Recently, the 5-HT$_{1D}$ receptor in the calf substantia nigra has been shown to be negatively-linked to adenylate cyclase using forskolin (Hoyer and Schoeffter, 1988). Inhibition of forskolin to elevate cAMP levels by the activation of a receptor is taken as evidence of a negatively-coupled arrangement. Interestingly the 5-HT$_{1D}$ receptor has not been identified in rodents and conversely the presence of 5-HT$_{1B}$ receptors has not been demonstrated in porcine, bovine or human brain tissue. It has been suggested that their similar

functional pharmacology (differing essentially in their respective sensitivities to certain beta-adrenoceptor antagonists) and their similar distribution in the brain (high concentrations are found in the basal ganglia and substantia nigra) reflects a biological equivalence (Waeber et al., 1988). Thus it would appear that the 5-HT$_{1B}$ subtype, also mediates inhibition of forskolin-stimulated adenylate cyclase activity in the rat substantia nigra (Bouhelal et al., 1988; Schoeffter and Hoyer, 1989). The opposite action on adenylate cyclase activity has been afforded to the putative 5-HT$_4$ receptor - evidence being currently in favour of a positive coupling arrangement (Dumuis et al., 1988).

Data obtained from *in vitro* release experiments (e.g. Middlemiss, 1985) supports the presence of a 5-HT autoreceptor situated presynaptically on 5-HT terminals which inhibits the stimulated release of 5-HT, its identity corresponding to the 5-HT$_{1B}$ receptor subtype (Engel et al., 1983). From the electrophysiological work of Aghajanian and colleagues as noted above there appears to be an autoreceptor located somatodendritically in the raphe nuclei. Autoradiographic localization of 5-HT$_{1A}$ receptors demonstrate a high concentration in the raphe nuclei, providing circumstantial evidence that identifies this subtype as the 5-HT cell body autoreceptor (Palacios et al., 1987). Behavioural evidence shows that where 5-HT is thought to have an inhibitory role, 8-OHDPAT has a stimulatory action, e.g. sexual activity (Ahlenius et al., 1981), feeding behaviour (Dourish et al., 1985; Bendotti and Samanin, 1986), body temperature control (Goodwin et al., 1985a) all of which support the 5-HT$_{1A}$ receptor as the cell body receptor in the raphe nuclei. However, the lack of specific antagonists of the functional effects of 8-OHDPAT combined with the

evidence from Middlemiss (1984) would suggest prudence before confirming an identity.

Unlike other 5-HT1 receptor subtypes, the 5-HT1c receptor appears to be coupled to phosphoinositol system (PI) (Conn et al., 1986). Chemical denervation of serotonergic neurons leads to a supersensitive phosphoinositide hydrolysis response to 5-HT1c receptor activation in the choroid plexus which would indicate a serotonergic input to the area (Conn et al., 1987) and it has been suggested that it may regulate CSF production (Sanders-Bush, 1987). Its considered role in CSF production is based on the observation that, the receptor appears to be located on the secretory epithelium of the choroid plexus (Yagaloff and Hartig, 1986), PI hydrolysis is involved in the regulation of other secretory and transport processes and that systemic administration of 5-HT depresses CSF formation (Maeda, 1983). On the basis of coupling to second messenger systems 5-HT1c displays more similarity to the 5-HT2 receptor than to other members of the 5-HT1 receptor family. In 1984 Conn and Sanders-Bush demonstrated the ability of specific 5-HT2 antagonists (ketanserin and pizotifen) to inhibit the 5-HT-induced increase in PI formation in the rat cerebral cortex. Regulation of phosphoinositol turnover by 5-HT2 receptor activation is thought to involve guanine nucleotides (Lyon et al., 1986). Further similarity between these two receptors is evident on consideration of their respective nanomolar affinities for 5-HT antagonists, in addition to the finding that radioligands used to label 5-HT2 sites can also be used to label 5-HT1c sites (Sanders-Bush and Breeding, 1988).

So far the receptors considered mediate their intracellular activities through a metabotropic mechanism, exploiting GTP-binding proteins, leading to changes in postsynaptic

cell excitability that are slow in onset and last at least for several hundreds of milliseconds. The 5-HT3 receptor is the first 5-HT receptor to be assigned an ionotropic function (Derkach et al., 1989). The ability of 5-HT to operate a cationic-channel indicates that it is capable of initiating immediate changes in membrane conductance.

The last word in biochemical receptor nomenclature will probably rest with molecular biology and the results of the latest receptor cloning experiments. So far the 5-HT$_{1A}$, 5-HT$_{1C}$ and 5-HT$_2$ receptors have been cloned and their amino acid sequence elucidated (Fargin et al., 1988; Lubbert et al., 1987; Julius et al., 1988; Pritchett et al., 1988). The structural homology displayed between the 5-HT$_2$ and 5-HT$_{1C}$ receptors lends further support to the argument that they should be reclassified as 5-HT$_{2A}$ and 5-HT$_{2B}$ respectively (Hartig, 1989). It remains to be seen whether this technique can resolve the high affinity/low affinity states of 5-HT$_2$ receptors proposed by Titler and colleagues and incorporate the remaining uncloned 5-HT receptors into a workable nomenclature.

1.3.5.2. Behavioural Correlates

Despite the undisputed importance of the radioligand-binding technique in identifying binding sites, it lacks the ability to determine the function of a binding site and therefore should not be used to define a receptor. Its usefulness is also limited in that it is incapable of distinguishing between agonist and antagonist molecules. With the rapidly multiplying number of 5-HT receptor subtypes it becomes increasingly necessary to identify functional binding sites in order to understand the physiological and pathophysiological implications of this

neurotransmitter. Analysis of behavioural measures specific to 5-HT receptor activation or blockade would allow a relatively easy means of characterizing receptors. The serotonergic system is fortunate in that there are several distinct behavioural effects induced by the activation of certain 5-HT receptors. Initial doubts concerning the confidence by which one might assign the 5-HT$_1$ binding site receptor status have been dispelled as a result of several functions being attributed to the activation of 5-HT$_{1\text{-like}}$ receptors. The grouping together of 5-HT$_1$ receptor subtypes under the title "5-HT$_{1\text{-like}}$" resulted from Bradley et al. (1986) who attempted to resolve the increasing controversy in the classification of 5-HT receptors. The principle of the exercise was to ensure that functional receptors were recognized as such and distinguished from binding sites. The study also realized the heterogeneity of 5-HT$_1$ binding sites and that functional correlates might well be attributed to them eventually. However, it was considered that the anomalies present at the time amongst these sites were insufficient to warrant granting a separate identity (i.e. 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$ etc.), hence the use of the term "5-HT$_{1\text{-like}}$". The classification of 5-HT receptors as 5-HT$_{1\text{-like}}$, 5-HT$_2$ and 5-HT$_3$, although still used, did not catch on universally and was not able to decrease "progress" on the identification of further 5-HT binding sites and their incorporation into the original Peroutka and Snyder classification system as A,B,C,D,E subtypes of the 5-HT$_1$ receptor.

Individual behaviours attributable to the activation of certain components of the serotonergic system are dealt with in greater detail in chapters 3 and 4. Briefly, the 5-HT syndrome first used by Corne et al. (1963) as a behavioural index of serotonergic activity, comprises several discrete symptoms thought to result from

postsynaptic 5-HT$_{1A}$ receptor activation (Hjorth et al., 1982; Tricklebank et al., 1985). These include reciprocal forepaw treading, hindlimb abduction, lateral headweaving, flat body posture, tremor, Straub tail, hyperreactivity and hyperactivity. An additional component, headtwitches (described as head-shakes or "wet-dog shakes" in the rat), arise from stimulation of the 5-HT$_2$ receptor (Ogren et al., 1979; Yap and Taylor, 1983; Niemegeers et al., 1983). Behavioural models associated with 5-HT$_1$ receptor activation include: hypothermia induced by 5-HT$_{1A}$ agonists; hyperphagia induced by 5-HT$_{1A}$ agonists or 5-HT$_{1B}$ agonists; facilitation of sexual behaviour by 5-HT$_{1A}$ agonists; and hypoactivity induced by putative 5-HT$_{1B}$ agonists (Lucki and Frazer, 1982; 1985). A recent report by Mittman and Geyer (1989), extended by Wing et al. (1990), demonstrates another potential behavioural model for 5-HT$_2$ receptor activity. The test is described as "potentiation of the normal neophobic reaction to a novel environment" and was first identified as a property of hallucinogenic compounds, e.g. LSD (Geyer and Light, 1979). Wing et al. (1990) have proposed that the reduction in exploratory behaviour exhibited by these compounds can be reversed by selective 5-HT$_2$ antagonists indicating a 5-HT$_2$ receptor mediating mechanism. In contrast, 5-HT$_{1\text{-like}}$ agonists might induce a suppression of activity in the novel environment but this is not blocked by the appropriate 5-HT$_2$ antagonists. This model was not able to detect the hallucinogen 5-methoxy-O-dimethyltryptamine (5MeODMT), a known non-selective 5-HT agonist, as a 5-HT$_2$ agonist since the reduction in exploratory activity was not reversed by ketanserin. Drug discrimination has been used to investigate both 5-HT$_1$ and 5-HT$_2$ receptor activation and is discussed in chapter 4.

Although 5-HT3 receptors have been implicated in a variety of behavioural disorders, most notably anxiety and psychosis (Cooper and Abbott, 1988), the identification of a distinct behavioural consequence to their activation which would act as a model has yet to be established. Electrophysiology and radioligand binding remain the most useful tools in their characterization.

(See Appendix, page 320 for table of central 5-HT receptor subtypes)

CHAPTER 2.

AIMS OF THE STUDY

2. AIMS OF THE STUDY

The aim of this study was to investigate the hypothesis that melatonin could affect the expression of 5-HT-mediated behaviours. This concept originated from the observation of their structural similarity, shared biosynthetic route, speculations from various authors that the mechanism of action might involve an interaction with the serotonergic system (e.g. Mess et al., 1983) and the more recent finding that 5-HT can affect a melatonin-induced behavioural pattern (Gaffori and Van Ree, 1985). It was quickly realized that the slight differences in molecular structure were sufficient to rule out any direct interaction with 5-HT receptors, but an indirect interaction could not be ruled out. In support of this, Dubocovich (1983, 1985) has shown that melatonin can selectively inhibit the calcium-dependant release of [^{3}H]-dopamine from rabbit and chicken retina through an action on its own receptor. The close relationship to circadian rhythms shared by both 5-HT and melatonin meant that an interaction between them might depend on the time of day and it was therefore decided to perform experiments, where possible, according to this factor.

It was the intention of the work described in chapter 5 to further explore the role of melatonin in the regulation of circadian rhythms, specifically, the circadian rhythm of locomotor activity. Moreover, it was thought that this would provide an opportunity to investigate whether the serotonergic system is involved in mediating the effects of melatonin on free-running circadian locomotor activity rhythms (see Redman et al., 1983).

The failure of an interaction between melatonin and 5-HT to be detected from the work conducted in chapter 3 led to the

investigation into the effects of melatonin on 5-HT-mediated conditioned behaviour, in the form of drug discrimination. It also provided a convenient means of assessing the ability of rats to discriminate between various doses of melatonin and saline. This work is described in chapter 4.

The work described in the final chapter was based on the hypothesis that melatonin, being released only at night, acts as a co-ordinator of the internal milieu of an animal such that it becomes adapted to night-appropriate behaviour. If this hypothesis were correct then an appropriately trained animal might be expected to make a dark-appropriate response in the presence of melatonin. The origin of this concept derived from the realization that all the physiological responses to melatonin appear to occur at a subconscious ("vegetative") level (see Herbert, 1989). The question as to whether melatonin might act as a discriminative stimulus directly was answered by the work discussed in chapter 4. It was reasoned that melatonin might express a discriminative cue in a slightly more subtle fashion, based on the relationship of environmental light and dark cues with temporal cues. An exteroceptive temporal/visual discriminative stimulus was employed using standard operant conditioning chambers with food reward.

CHAPTER 3.

MELATONIN AND 5-HT

3. MELATONIN AND 5-HT

3.1. Evidence for the Physiological/Pharmacological Interaction of 5-HT and Melatonin

The common biosynthetic pathway shared by 5-HT and melatonin and hence their similar molecular structures provided the initial impetus to justify investigation as to whether these two compounds in some way interacted. One of the earliest studies to support this concept was produced by Anton-Tay et al. (1968) who reported an increase of serotonin content in the hypothalamus and midbrain of rats administered melatonin, though this has not proved to be a consistent finding (e.g. Holmes and Sugden, 1982). As discussed in Chapter 1, several recently published reports question the passive role of 5-HT as simply a precursor in melatonin synthesis (Aloyo and Walker, 1987; Aloyo and Walker, 1988; Sugden, 1989). In addition, several workers have proposed that the effects of melatonin are mediated via the serotonergic system (e.g. Wurtman and Anton-Tay, 1969; Kovacs et al., 1974; Trentini et al., 1979). Despite this apparent interest there have been few reports published specifically aimed at identifying a link between melatonin and 5-HT in a discipline other than synthetic biochemistry.

In the periphery it has been demonstrated that high concentrations of melatonin inhibit the contractile response to 5-HT in certain isolated smooth muscles (Satake et al., 1986). Kamberi (1974) described evidence for an inhibitory effect of 5-HT on the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) which is mimicked by melatonin. Others have reported a positive action on the cyclic release of gonadotropins and prolactin secretion by 5-HT (see review by Trentini, 1979). Evidence derived

from radioligand binding studies involving both 5-HT receptor ligands and [^{3}H]-melatonin or 2-[^{125}I]-iodomelatonin indicate that their small structural differences are sufficient to confer negligible affinity for each others putative binding sites (Dubocovich and Takahashi, 1987; Morgan and Williams, 1989). Pickering and Niles (1990), used more selective 5-HT radioligands, and also failed to evince inhibition of binding by 2-[^{125}I]-iodomelatonin. An interesting exception, however, was the mixed 5-HT$_{1A}$/5-HT$_{1B}$ receptor agonist, RU24969, which possessed appreciable affinity for binding sites labelled by 2-[^{125}I]-iodomelatonin in the hypothalamus presumably due to its structure being based around an indole nucleus. This suggests that an interaction, if existing between the serotonergic system and melatonin, would be expressed via an indirect mechanism. Interestingly, the choroid plexus, which lies at the base of the pineal stalk, is reported to possess a low concentration of melatonin binding sites as identified by 2-[^{125}I]-iodomelatonin (Vanecek, 1988) and a high concentration of 5-HT$_{1c}$ binding sites (Pazos et al., 1984). Gaffori and Van Ree (1985) have advanced the most compelling support for an interrelationship between melatonin and 5-HT. They were able to show that injection of small amounts of melatonin directly into the nucleus accumbens elicited a distinctive change in rat behaviour. This "melatonin-induced" behaviour was characterized by an increase in grooming and sniffing displacement-type behaviour (Silverman, 1978) and a reduction in locomotor and rearing activity. Recognized dopaminergic agonists and antagonists were unable to affect the action of melatonin but 5-HT blocked the behaviour and it was reproduced by non-selective 5-HT antagonists.

There has been much speculation in the literature over 5-HT and its possible role in mediating the action of melatonin. It was decided to use recognized behavioural models of 5-HT receptor activation to investigate the potential of a modulatory role for melatonin in the serotonergic system. These models and any related actions of melatonin are considered below.

3.2. Behavioural Models of 5-HT Receptor Activation

3.2.1. The Headtwitch

The headtwitch is a component of the 5-HT behavioural syndrome that is considered to be mediated by activation of the 5-HT_2 receptor. Early experiments investigating the 5-HT behavioural syndrome induced it by the administration of a monoamine oxidase inhibitor (MAOI) in conjunction with a precursor of 5-HT (L-tryptophan or 5-HTP) but has since been reproduced with other compounds capable of activating 5-HT receptors. After the classification of 5-HT receptors into 5-HT_1 and 5-HT by Peroutka and Snyder (1979a) it was thought that manifestation of the whole syndrome was attributable to the activation of 5-HT_2 receptors (Peroutka et al., 1981), however attention was focused on 5-HT_1 receptors after Lucki and Frazer (1982a) reported that chronic antidepressant treatment which down-regulated 5-HT_1, but not 5-HT_2 receptors inhibited the ability of 5MeODMT to elicit the syndrome. Furthermore, the novel 5-HT_2 receptor antagonist, ketanserin, failed to block the appearance of the syndrome induced by 5-MeODMT in contrast to the non-selective anatgonist metergoline (Lucki et al., 1984). The introduction of 8-OHDPAT in 5-HT behavioural studies and its ability to induce the syndrome, identified the 5-HT_{1A} receptor as the probable mediator (Hjorth et al., 1982; Tricklebank et al.,

1985). Putative selective 5-HT$_{1B}$ receptor agonists (e.g. 1-[3-(trifluoromethyl)phenyl] piperazine, TFMPP; or 1-[3-chlorophenyl]piperazine, mCPP) do not readily produce the syndrome (Lucki and Frazer, 1982b). Inhibiting 5-HT synthesis using the tryptophan hydroxylase inhibitor, pCPA, depleting 5-HT stores with reserpine or destroying 5-HT neurons using 5,7-dihydroxytryptamine (5,7-DHT) fails to prevent the syndrome induced by 8-OHDPAT suggesting a postsynaptic receptor as its mediator (Tricklebank et al., 1985). Closer examination of the effects of ketanserin and other 5-HT$_2$ antagonists on the behavioural syndrome reveal that they are capable of blocking a single component, that of headtwitches (Ogren et al., 1979; Yap and Taylor, 1983; Niemegeers et al., 1983). This would suggest that this symptom is induced through the activation of 5-HT$_2$ receptors. Certain hallucinogenic compounds known to interact with 5-HT$_2$ receptors are capable of eliciting headtwitches, e.g. LSD, mescaline and DOB (Vetulani et al., 1980; Niemegeers et al., 1983; Lucki et al., 1984). The ability of 5-HT antagonists to block the headtwitch response correlates with their affinity for 5-HT$_2$ binding sites in the frontal cortex (Ogren et al., 1979; Niemegeers et al., 1983), though it is thought that the behaviour is actually mediated by 5-HT receptors located in the brain stem (Bedard and Pycock, 1977; Lucki and Minugh-Purvis, 1987).

In this series of experiments the headtwitch response was used as a model for 5-HT$_2$ receptor activation with the aim of discovering whether it was influenced by melatonin. In addition, chronic administration of certain antidepressant drugs inhibits the head-shaking behaviour in rats if tested during drug treatment (Lucki and Frazer, 1985), an effect possibly caused by down-regulation of 5-HT$_2$ receptors (Peroutka and Snyder, 1980). It was

thought that melatonin might exert a similar effect in mice and therefore this hypothesis was also tested.

3.2.2. Hypothermia

The hypothermic response induced by 8-OHDPAT was used as a model for 5-HT_{1A} receptor activation. The serotonergic system has long been associated with a regulatory function in the maintenance of deep body temperature in homeothermic animals although the reaction to serotonergic drugs is highly dependent on dose, species and environmental conditions (Green and Goodwin, 1987). Activation of 5-HT_2 receptors would appear to elicit hyperthermia since temperature increases induced by certain 5-HT agonists, e.g. quipazine, mCPP and high doses of 5MeODMT are inhibited by pretreatment with 5-HT_2 antagonists (Gudelsky et al., 1986). In contrast, 8-OHDPAT, ipsapirone and low doses of 5MeODMT induce hypothermia (Goodwin and Green, 1985; Goodwin et al., 1985, 1986, 1987; Gudelsky et al., 1986). 8-OHDPAT-induced hypothermia can be attenuated by prior administration of ipsapirone, supporting evidence that this compound acts as a partial agonist at 5-HT_1 receptors (Goodwin et al., 1986). The hypothermic response to 8-OHDPAT in rats can be blocked by putative 5-HT_{1A} antagonists, e.g. (-)-propranolol and (-)-pindolol, but not selective 5-HT_2 antagonists (Goodwin and Green, 1985; Gudelsky et al., 1986). In mice these same 5-HT_{1A} antagonists fail to block 8-OH- DPAT-induced hypothermia (Goodwin and Green, 1985). Interestingly, quipazine and the butyrophenone haloperidol are capable of attenuating the response in mice (Goodwin et al., 1985a). Since flupenthixol is without effect on 8-OHDPAT-induced hypothermia it would seem reasonable that haloperidol is acting at a butyrophenone-sensitive

site rather than via the dopaminergic system (Goodwin et al., 1985a), which Goodwin and Green (1985) suggest could be the 5-HT$_2$ receptor. It would appear that the hypothermic response to 8-OHDPAT results from the activation of presynaptic 5-HT$_{1A}$ receptors since it is abolished if 5-HT synthesis is inhibited using pCPA or 5-HT neurons are destroyed using 5,7-DHT (Goodwin et al., 1985a, 1987).

Melatonin and the pineal gland have also been implicated in the regulation of body temperature. John et al. (1978) reported elevated body temperature in pinealectomized pigeons in comparison to their intact controls. This hyperthermia was blocked by the subcutaneous implantation of melatonin in beeswax pellets. Pinealectomy abolished the circadian rhythm of body temperature of house sparrows housed in DD (Binkley et al., 1971). Binkley et al. (1971) also reported that injection of melatonin into house sparrows elicited a 4.7°C decrease in body temperature. The published effects of melatonin on body temperature in mammals are few and contradictory. Large doses of melatonin (50-100mg) appear to reduce body temperature in mice (Arutyunyan et al., 1964) though this may be due to a generalized reduction in activity resulting from its hypnotic effect (Sugden, 1980). In contrast a hyperthermic effect on the body temperature in rats was observed by Fioretti et al. (1974) using smaller doses (4-8mg/kg). Barchas et al. (1964) found no evidence that melatonin influenced rabbit body temperature.

It was clear that melatonin might elicit an effect on 8-OHDPAT-induced hypothermia which was not due to any form of active melatonin-5-HT interaction. Thus experiments were conducted to establish whether melatonin at moderate doses influenced body temperature in mice, prior to investigating the action of melatonin on 5-HT$_{1A}$ receptor activation.

3.2.3. Hyperphagia

In the past, activation of the serotonergic system was generally considered to inhibit feeding behaviour. Drugs that increase the synaptic availability of 5-HT or activate 5-HT receptors reduce food intake. Thus the 5-HT releasing agent, fenfluramine, the 5-HT reuptake inhibitor, fluoxetine and the 5-HT agonists quipazine and 5-HTP all inhibit feeding (Blundell and Lesham, 1975; Goudie et al., 1976; Samanin et al., 1977). Moreover, the "classical" 5-HT antagonists (e.g. cyproheptadine) promote food intake (Baxter et al., 1970). The comfort of this theory was briefly disturbed by the report that the novel 5-HT agonist 8-OHDPAT, which was a potent and selective ligand for 5-HT$_{1A}$ receptors (Middlemiss and Fozard, 1983), induced feeding in non-deprived rats (Dourish et al., 1985). It has since been shown that hyperphagia is the result of 8-OHDPAT acting on 5-HT$_{1A}$ autoreceptors located in the raphe nuclei to reduce serotonergic activity (Dourish et al., 1986a, 1986b; Hutson et al., 1986) in contrast with the 8-OHDPAT-induced behavioural syndrome which is produced by activation of postsynaptic 5-HT$_{1A}$ receptors (Tricklebank et al., 1984). Stimulation of these autoreceptors is thought to inhibit 5-HT synthesis since 8-OHDPAT fails to elicit feeding in rats pretreated with the 5-HT synthesis inhibitor pCPA (Dourish et al., 1986a). This is consistent with the inhibitory role played by the serotonergic system in feeding behaviour.

8-OHDPAT-induced hyperphagia was chosen as an additional model for 5-HT$_{1A}$ receptor activation to investigate the possible influence of melatonin which has not been reported to exert a significant effect on feeding behaviour.

3.2.4. Locomotor Activity

The influence of melatonin on 5-HT$_{1B}$ receptor-mediated behaviour was investigated using RU24969-induced hyperactivity as a putative model for 5-HT$_{1B}$ receptor activation. As with feeding, activation of the serotonergic system has usually been associated with an inhibitory effect on locomotor activity. This is derived from studies that demonstrate depletion of central 5-HT (e.g. caused by vesicular depletion, inhibition of synthesis or destruction of 5-HT neurons) induce hyperactivity (Gerson and Baldessarini, 1980) and the intracerebroventricular (i.c.v.) administration of 5-HT reduces locomotor activity (Green et al., 1976). This effect is thought to be partly mediated by the dopaminergic neurons of the mesolimbic system since depletion of 5-HT potentiates hyperactivity induced by dopaminergic agonists, e.g. amphetamine, (Lucki and Harvey, 1979) and i.v. injection of 5-HT inhibits this stimulated locomotor activity response to amphetamine (Warbritton et al., 1978). In addition, RU24969 has been shown to enhance the activity of the mesolimbic system (Oberlander, 1983), despite having no affinity for dopamine receptors (Tricklebank, 1986). Furthermore, non-sedative doses of haloperidol have been reported to inhibit RU24969-induced hyperactivity (Green et al., 1984). Thus it would appear possible that RU24969 acts by reducing an inhibitory serotonergic influence on dopamine neurons (Carli et al., 1988). RU24969 has recently been shown to possess similar affinity for both the 5-HT$_{1A}$ and 5-HT$_{1B}$ binding sites (Hamon et al., 1986; Tricklebank et al., 1986). However, the main behavioural symptoms of RU24969 administration are hyophagia and hyperactivity (Green et al., 1984). In contrast, 8-OHDPAT induces components of the behavioural syndrome, increased sexual behaviour, hyperphagia and hypothermia

(Ahlenius et al., 1981; Tricklebank et al., 1986; Dourish et al., 1985; Bendotti and Samanin, 1986). This would suggest that RU24969-induced hyperactivity results from an interaction at 5-HT$_{1B}$ receptors although it is possible that a difference in brain distribution of 5-HT$_{1A}$ and 5-HT$_{1B}$ binding sites might account for the differing behavioural effects of 8-OHDPAT and RU24969 (Pazos and Palacios, 1985; Carli et al., 1988).

Hypoactivity has also been reported as a suitable model for 5-HT$_{1B}$ receptor activation in contrast to hyperactivity. This is derived from studies that report hypoactivity as the major effect of the putative selective 5-HT$_{1B}$ agonists TFMPP and mCPP in unhabituated rats exposed to a novel environment (Lucki and Frazer, 1982b, 1985). The response is blocked by pretreatment with non-selective 5-HT antagonists (e.g. metergoline) but not selective 5-HT$_2$ antagonists (e.g. ketanserin) suggesting that it is mediated by 5-HT$_{1-like}$ receptors (Lucki and Frazer, 1985). Recently it has been reported that low doses of RU24969 elicit hypoactivity in the mouse which appeared to be due to reduced 5-HT release as a result of activation of the 5-HT$_{1B}$ autoreceptor (Clark et al., 1988). It was decided to investigate this effect in rats to ascertain whether it might be a more appropriate model for 5-HT$_{1B}$ receptor activation than RU24969-induced hyperactivity.

Information on whether melatonin has a direct effect on locomotor activity is not clear although several reports have been published documenting the effect of melatonin on the circadian rhythm of locomotor activity in various species (e.g. Underwood and Harless, 1985; Underwood, 1986; Redman et al., 1983; Cassone et al., 1986a, 1986b). This is considered in greater detail in chapter 5. It has been shown that continuous infusion of melatonin from i.p.

placed silastic capsules reduces the total amount of perch-hopping behaviour per 24 h in sparrows (Hendel and Turek, 1978). In addition wheel-running activity in rats is depressed by melatonin in food-deprived rats (Wong and Whiteside, 1968). Both of these studies, however, were concerned with the circadian pattern of activity rather than an immediate pharmacological effect. Considering its putative hypnotic properties large doses might be expected to elicit hypoactivity. As mentioned above Gaffori and Van Ree (1985) have reported a reduction in locomotor activity after an injection of melatonin into the nucleus accumbens of rats which appears to be influenced by 5-HT through an unknown mechanism. Peripheral administration of melatonin has tended to yield negative results (e.g. Kastin et al., 1973; Kovacs et al., 1974).

3.3. Melatonin, 5-HT and Circadian Rhythms

Several parameters of central 5-HT function display a circadian variation. 5-HT concentrations in the brain express a circadian rhythm with a zenith at approximately mid-light (ML) (Albrecht et al., 1956; Hillier and Redfern, 1977). Conversely the circadian rhythm of 5-HT release as determined by intracerebral analysis peaks during the dark phase (Martin and Marsden, 1985). This is in phase with the circadian rhythm of 5-HT reuptake (Wirz-Justice et al., 1983). [^{3}H]-Spiperone binding in the frontal cortex also displays a 24 h variation (Wirz-Justice, 1987), peaking at ML. This suggestion of 5-HT$_2$ receptor density variation is supported by Moser and Redfern (1985a) who demonstrated that the 5-HT$_2$ receptor mediated headtwitch behaviour in mice expresses a circadian rhythm with a maximum at ML. 5-HT$_{1A}$ receptor mediated behaviour (e.g. 5-HT behavioural syndrome and 8-OHDPAT-induced

hypothermia) does not undergo a circadian variation (Moser, 1986). Since melatonin has been shown to influence the expression of circadian rhythms in a variety of species it was decided to conduct these experiments at pre-determined time-points of the LD cycle. Melatonin has often been described as "the hormone of darkness" and it was therefore hypothesized that any interaction with the serotonergic system might be the result of melatonin inducing dark-appropriate responding.

Experiments that were of relatively short duration (8-OHDPAT-induced hypothermia and headtwitches) were performed at four times: end-of-light (EOL), mid-dark (MD), end-of-dark (EOD) and mid-light (ML). The "end of" light or dark was taken as the last two hours of the phase. Thus no experiment was conducted which would involve the animals experiencing a sudden change of phase during its course. The feeding and activity experiments were of longer duration and were performed at two periods of the LD cycle. These experiments were conducted over the course of five hours of which the mid-point was approximately MD or ML. The effect of chronic melatonin treatment on L-5HTP-induced headtwitches was conducted at only one time point.

3.4. Materials and Methods

3.4.1. Animals

Male CFLP mice (Animal House, Bath University strain) weighing between 25 and 40g (unless stated otherwise), housed in groups of 20-25 under normal lighting conditions (12:12 LD cycle; lights on 06:00 h) were used in all mouse experiments. Male Wistar rats (Animal House, Bath University strain) weighing between 250 and 400g, housed in groups of 4-6 in standard plastic cages, measuring

520 x 350 x 170mm, under a 12:12 LD lighting regimen were used in all rat experiments. Food and water (Labsure CRM diet) were available *ad libitum* except where indicated otherwise.

3.4.2. Drugs

All drugs were dissolved or suspended in normal saline by sonication and administered i.p. unless otherwise stated using a dose-volume of 5ml/kg. Control animals received the same amount of drug vehicle. Drugs used were as follows: Melatonin (Sigma); 5-Methoxy-dimethyltryptamine (5MeODMT, Sigma); 5-Hydroxy-L-tryptophan (L-5HTP, Sigma); 8-dihydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT, gift from Dr. J Stolz, University of Bath), injected s.c. using a dose-volume of 2.5ml/kg; 5-methoxy-3(1,2,3,6-tetrahydro-pyridin-4-yl) 1H indole (RU24969, Roussell); and carbidopa (gift from M.S.D.).

3.4.3. Methods

3.4.3.1. Investigation into the effect of melatonin on headtwitches induced by 5-HT agonists.

One mouse was placed into one half of a cage measuring 330 x 130 x 160mm divided into two halves by a wooden block 2 min after an injection of 5MeODMT (8mg/kg) or 20 min after an injection of L-5HTP (160mg/kg). A second mouse was put into the other half thereby enabling two mice to be observed for 2 min simultaneously. Mice that received L-5HTP were pretreated 20 min before with carbidopa (50mg/kg). A range of melatonin doses (0, 0.05, 0.2, 0.8, 1, 4, 8, 16, 32mg/kg) and pre-treatment times (10, 20, 30 min) were used. Control animals received the same amount of drug vehicle. The effect of chronic treatment with melatonin (0.05, 0.2 and 0.8mg/kg)

was also investigated on headtwitches induced by L-5HTP. Mice weighing 15-25g at the start of the experiment were injected with melatonin daily over a 15 day period. They were weighed each day to ensure that any changes in the injection volume could be accomodated when necessary. On the 16th day the mice were pretreated with carbidopa, followed 10 min later by the same dose of melatonin as they had received chronically. 10 min after the melatonin injection the mice received L-5HTP and observed, as described above, 20 min later. The effect of melatonin (1mg/kg) on headtwitches induced by 5MeODMT (8mg/kg) at four time-points of the LD cycle was examined. The points chosen were ML, EOL, MD and EOD.

3.4.3.2. Investigation into the effect of melatonin on 8-OHDPAT-induced hypothermia.

Mice weighing 25-30g were used in all temperature experiments. Initial dose-response and time-response curves to 8-OHDPAT and melatonin were constructed with rectal temperatures being taken immediately before injection and at predetermined times after injection using a vaseline-lubricated probe inserted 2.5cm into the rectum. Animals used for investigating the effects of 8-OHDPAT-induced hypothermia had their rectal temperature measured four times: 15 min before their first injection (8-OHDPAT, 0.5mg/kg), immediately before the first injection, immediately before their second injection (melatonin, 1mg/kg) and finally 15 min later. Temperatures were measured using a vaseline-lubricated probe inserted 2.5cm into the rectum, the mice being lightly restrained. All measurements were taken at room temperature (20-25°C). Antagonism experiments were performed at four time points: ML, EOL, MD and EOD. Control animals received the same amount of drug

vehicle.

3.4.3.3. Investigation into the effect of melatonin on 8-OHDPAT-induced hyperphagia.

Mice weighing between 42 and 47g, and rats weighing between 300 and 400g were used for this series of experiments. Animals were taken from their home cage and placed into individual observation plastic cages, measuring 420 x 250 x 120mm for mice and 420 x 260 x 150mm for rats, for 2 h before the experiment was begun. This allowed them to habituate themselves with the experimental cages and also with food pellets (Labsure CRM diet) located on the cage floor. Water was available from a standard water bottle. Just prior to the start of the experiment the animals were removed from the cages and returned to their home cage. The experimental cages were then cleaned and a weighed amount of food was placed onto the cage floor. As the animals were put back into their respective experimental cages they received an injection of drug. The effect of melatonin on feeding was assessed using 0.1, 1 and 10mg/kg in both mice and rats. A dose-response curve to 8-OHDPAT was constructed using rats. Animals used to assess the effect of melatonin on 8-OHDPAT-induced hyperphagia were given 8-OHDPAT (0.25mg/kg) at the start of the experiment and then 15 min later received melatonin (1mg/kg). After the experiment food remnants were collected and weighed. Control animals received the same amount of drug vehicle.

3.4.3.4. Locomotor Activity Experiments

3.4.3.4.1. Investigation into the effect of melatonin on RU24969-induced hyperactivity.

Mice weighing between 35 and 45g were housed individually in plastic cages measuring 520 x 350 x 170mm placed on top of Animex

locomotor activity detectors. These detectors were housed in sound and light attenuating environmental cabinets (see Fig. 5.1, chapter 5) which were equipped with their own lighting and ventilation system (see chapter 5, section 5.2.3. for further details). The number of counts was collected and displayed, via a BBC Master computer, on a monitor screen using a short-term locomotor activity program (Marshall et al., 1985). The data was stored on computer disc as counts/min and for all experiments the total number of counts measured over 2 h following injection of the drug were recorded. A dose-response curve to RU24969 was constructed and potential melatonin-induced effects on locomotor activity were investigated at two doses (1 and 9mg/kg). Interaction experiments were performed with the injection of melatonin (1mg/kg) being given immediately after that of RU24969 (5mg/kg). This pretreatment time for melatonin was chosen because preliminary experiments established that hyperactivity induced by RU24969 began approximately 30 min after injection of the drug.

3.4.3.4.2. Assessment of the ability of low-dose RU24969 to induce hypoactivity in rats

Rats weighing between 250 and 350g were housed individually in a rectangular plastic cage measuring 900 x 600 x 350mm as described in chapter 5, section 5.2.3. (see Figs. 5.3 and 5.4). Activity was assessed by means of light-beam breakage and recorded as counts/2 min using specifically designed software (Marshall et al., 1985). Animals were allowed to habituate to the cage for 2 h before receiving an injection of drug. They were then returned to the cage and activity recorded for the following 2 h. The experiment was performed over the MD period.

3.5. Results

3.5.1. Melatonin and headtwitches

Melatonin neither elicited any headtwitches nor influenced the number of headtwitches induced by either of the 5-HT agonists used at any of the time points or dosages used. Fig. 3.1a and 3.1b depict the dose-response curves obtained with 5MeODMT and L-5HTP respectively. Fig. 3.2 compares a range of melatonin doses (0.2, 1.0, 8.0, 32.0mg/kg, 20 min pretreatment time) with 5MeODMT (8mg/kg, 2 min pretreatment time) and L-5HTP (80mg/kg, 20 min pretreatment time) in their ability to induce headtwitches in mice. As can be seen from Fig. 3.3 chronic melatonin therapy also had no effect on the number of headtwitches induced by L-5HTP. Fig. 3.4 depicts the effect of melatonin (1mg/kg, 20 min pretreatment time) on 5MeODMT-induced headtwitches over four time points in the LD cycle (12:12).

3.5.2. Melatonin and temperature

Fig. 3.5 shows the dose-response curve for 8-OHDPAT-induced hypothermia. Melatonin failed to affect mouse rectal temperature at any dose employed (see Table 3.1) and also failed to influence hypothermia induced by 8-OHDPAT (0.5mg/kg) at any of the time points over the LD cycle (1mg/kg, 15 min pretreatment time) as shown by Fig. 3.6.

3.5.3. Melatonin and feeding

The ability of 8-OHDPAT to induce hyperphagia was confirmed (see Fig. 3.7) but melatonin failed to influence feeding in both mice and rats (see Table 3.2) and was ineffective at modifying the hyperphagia induced by 8-OHDPAT in rats at both the ML

and MD periods investigated (see Fig. 3.8).

3.5.4. Locomotor Activity Experiments

3.5.4.1. Melatonin and RU24969-induced hyperactivity

The dose-response curve to RU24969 is shown in Fig. 3.9. Melatonin (1mg/kg) did not influence mouse locomotor activity alone and was without effect on RU24969-induced hyperactivity when given immediately after the injection of RU24969 (5mg/kg) during either the dark or the light phase (see Fig. 3.10).

3.5.4.2. RU24969 and hypoactivity

The ability of low doses of RU24969 to induce hypoactivity in mice was not confirmed in the rat. Fig. 3.11 shows the mean total activity over 30 min after receiving various doses of the drug up to the level when hyperactivity became discernible.

Table 3.1. Effect of melatonin on rectal temperature in the mouse at mid-light using different pre-treatment times. ("+" or "-" indicate increase or decrease, respectively, in rectal temperature; n=6).

Dose (mg/kg)	Pretreatment time (min)	Mean change in rectal temperature (°C) +/- s.e.m.	
0	10	0	0.12
0.1	10	+ 0.1	0.14
1.0	10	- 0.04	0.13
10.0	10	+ 0.18	0.17
0	20	+ 0.05	0.13
0.1	20	+ 0.13	0.08
1.0	20	+ 0.02	0.05
10.0	20	- 0.02	0.12
0	30	- 0.04	0.16
0.1	30	+ 0.05	0.06
1.0	30	0	0.14
10.0	30	- 0.04	0.08

Table 3.2. Effect of melatonin on food intake of mice and rats at mid-light (ML) and mid-dark (MD). (n=6).

Animal	Dose (mg/kg)	Time-of-day	Mean quantity of food eaten in 2 h (g) +/- s.e.m.	
Mouse	0	ML	0.017	0.01
	0.1		0.017	0.01
	1.0		0.04	0.02
	10.0		0.014	0.01
Mouse	0	MD	1.14	0.12
	0.1		1.02	0.06
	1.0		0.82	0.09
	10.0		0.95	0.06
Rat	0	ML	0.83	0.26
	0.1		0.41	0.21
	1.0		0.48	0.20
	10.0		0.54	0.29
Rat	0	MD	2.44	0.54
	0.1		3.32	0.32
	1.0		2.80	0.62

Fig. 3.1. Dose response curves showing the induction of headtwitches in mice by (a) L-5HTP and (b) 5MeODMT. (n=5-7, mean ± s.e.m.).

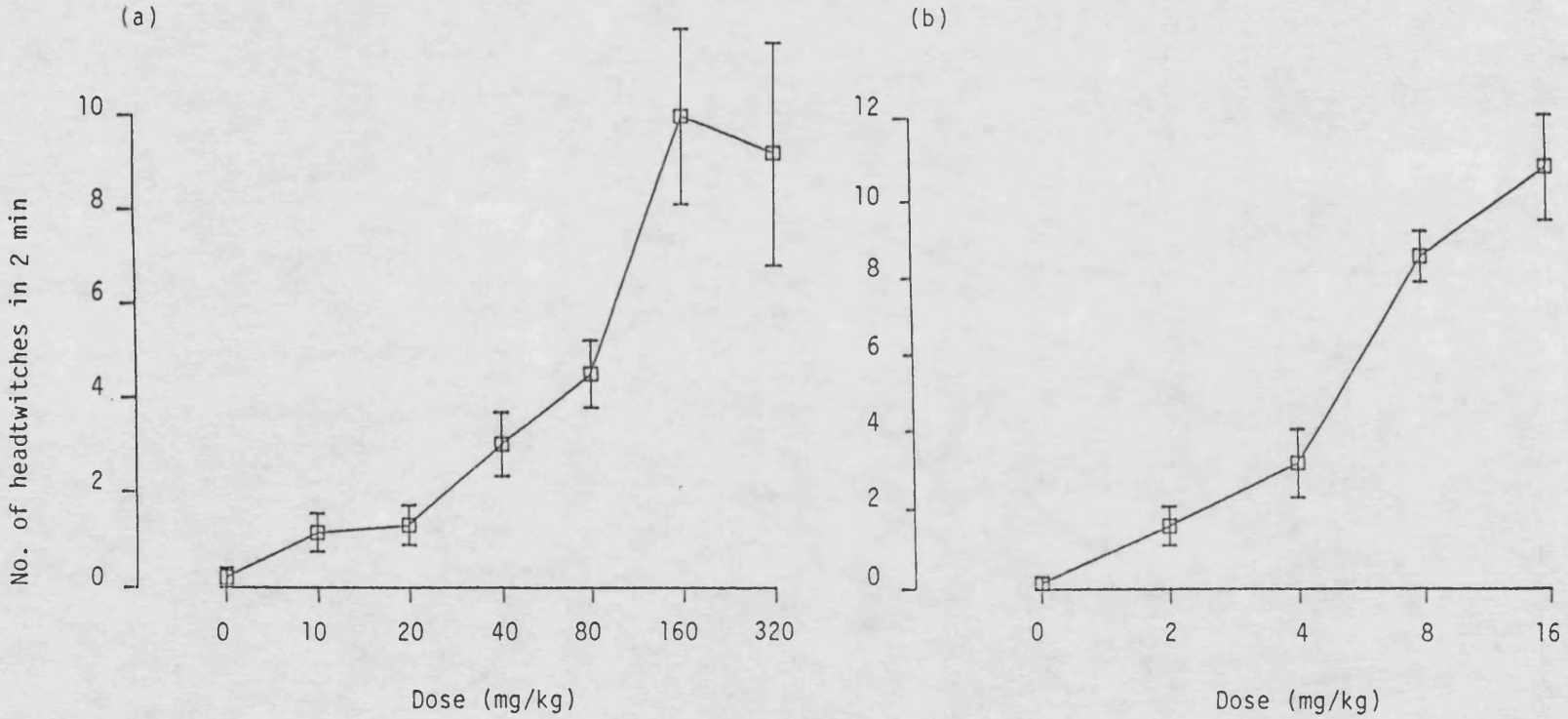
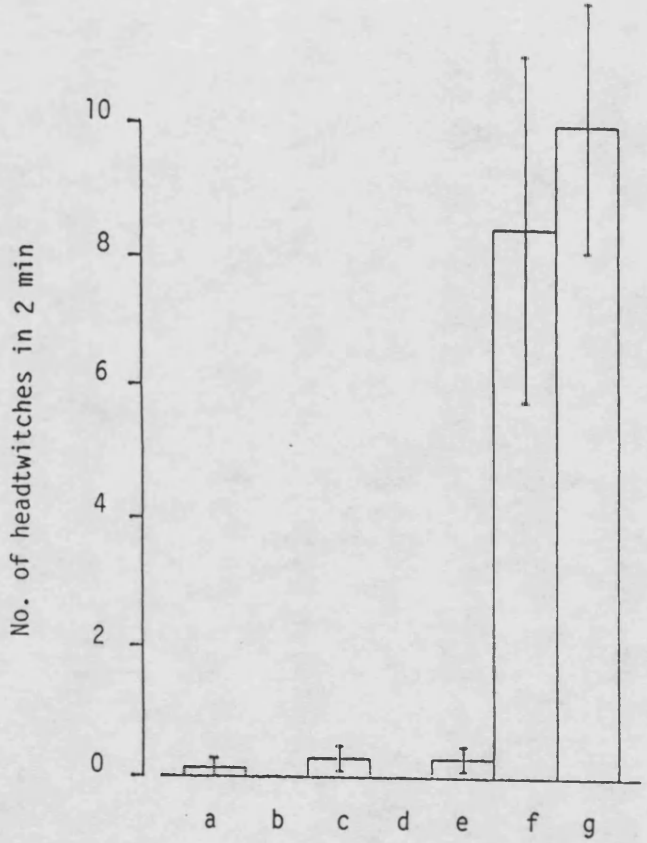


Fig. 3.2. Headtwitches induced by melatonin, 5MeODMT and L-5HTP. (n=5-7, mean ± s.e.m.).



Key:

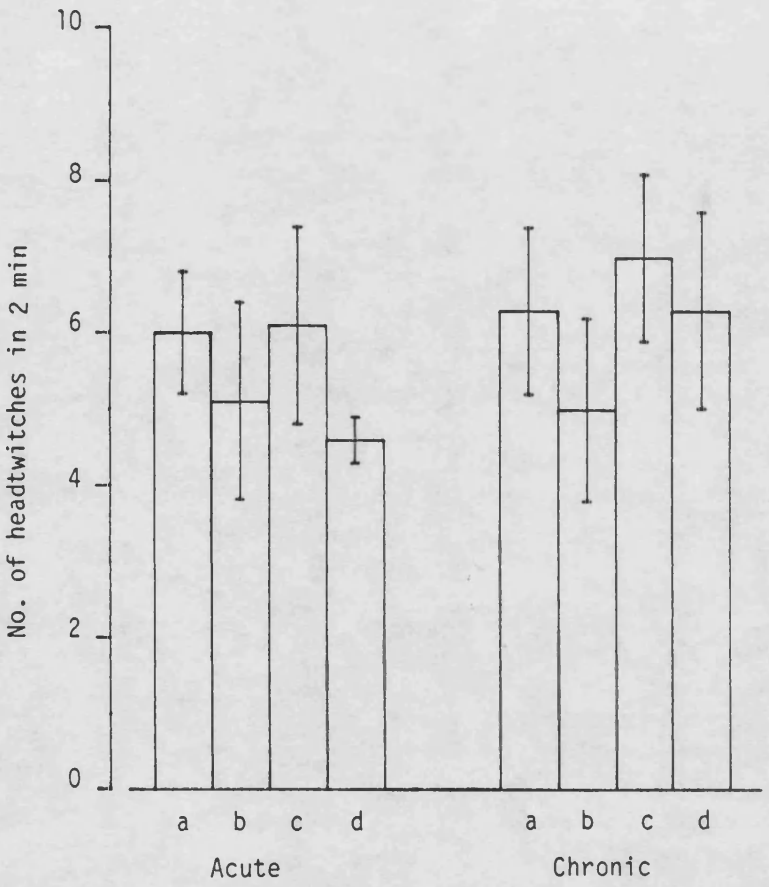
a = vehicle

b-e = 0.2, 1.0, 8.0 and 32.0mg/kg melatonin repectively

f = 5MeODMT (8mg/kg)

g = L-5HTP (80mg/kg)

Fig. 3.3. Comparison of acute melatonin and chronic melatonin treatment on L-5HTP-induced headtwitches in mice. (n=6-7, mean ± s.e.m.).



Key:

a = vehicle: L-5HTP (80mg/kg)

b = melatonin (0.05mg/kg): L-5HTP (80mg/kg)

c = melatonin (0.2mg/kg): L-5HTP (80mg/kg)

d = melatonin (1.0mg/kg): L-5HTP (80mg/kg)

All animals were given carbidopa (25mg/kg) i.p. 20 min before receiving L-5HTP.

Fig. 3.4. Effect of melatonin on 5MeODMT-induced headtwitches in mice over 24 hours. (n=6, mean ± s.e.m.).

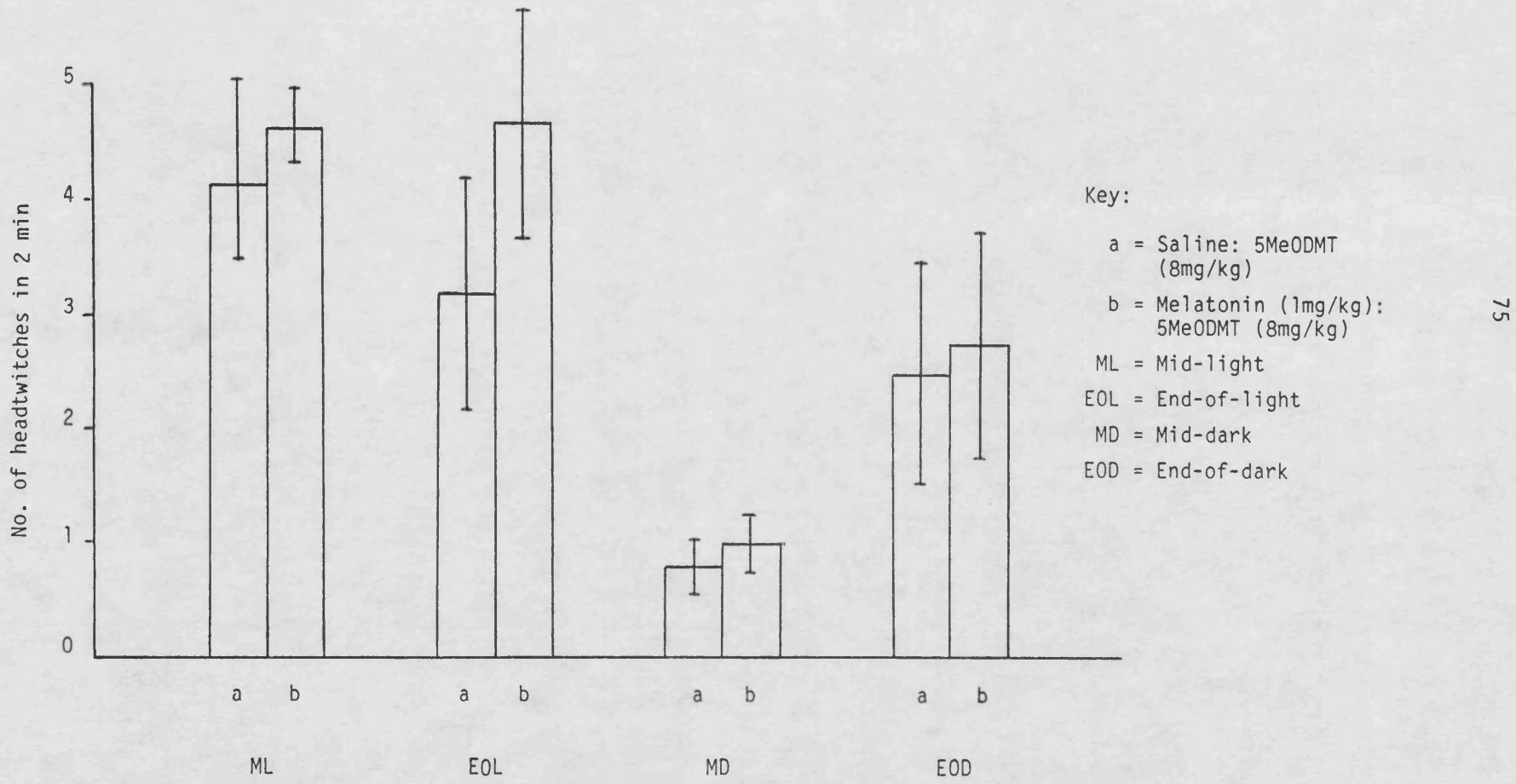


Fig. 3.5. Dose-response curve showing the induction of hypothemia by 8-OHDPAT in mice. (n=6, mean ± s.e.m.).

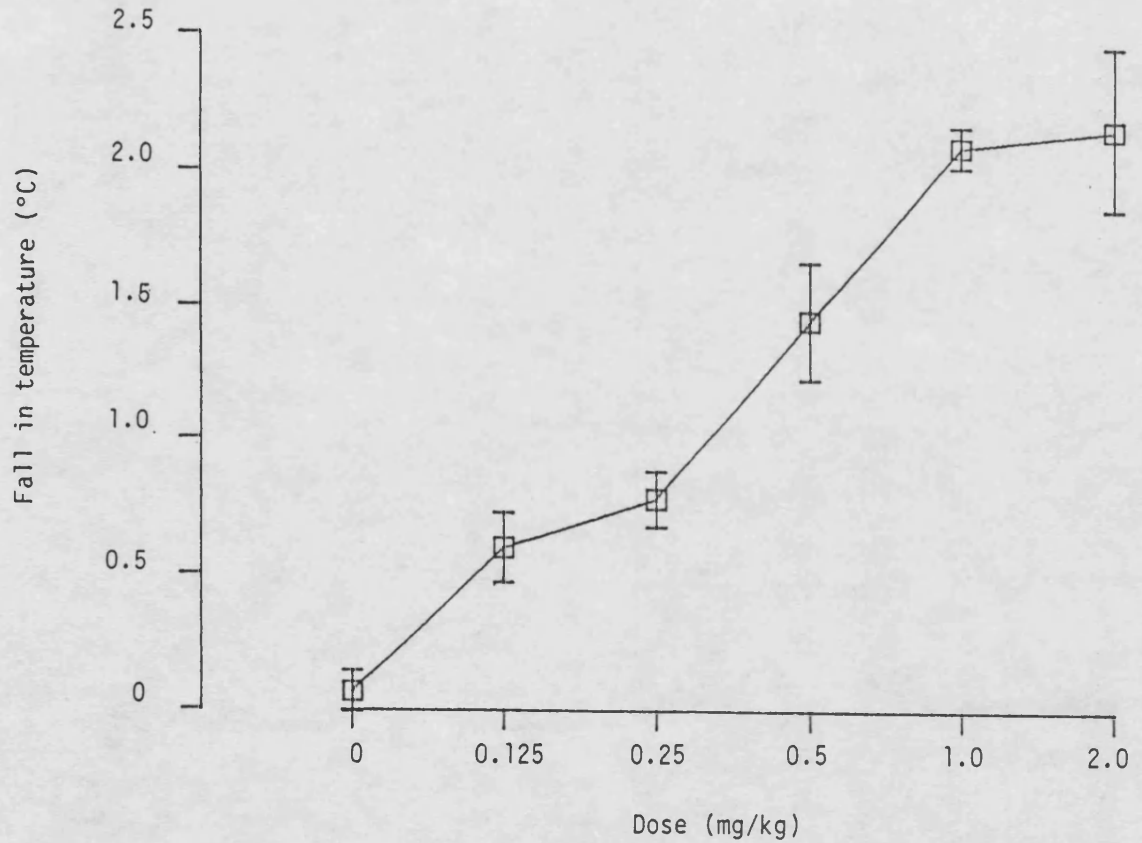
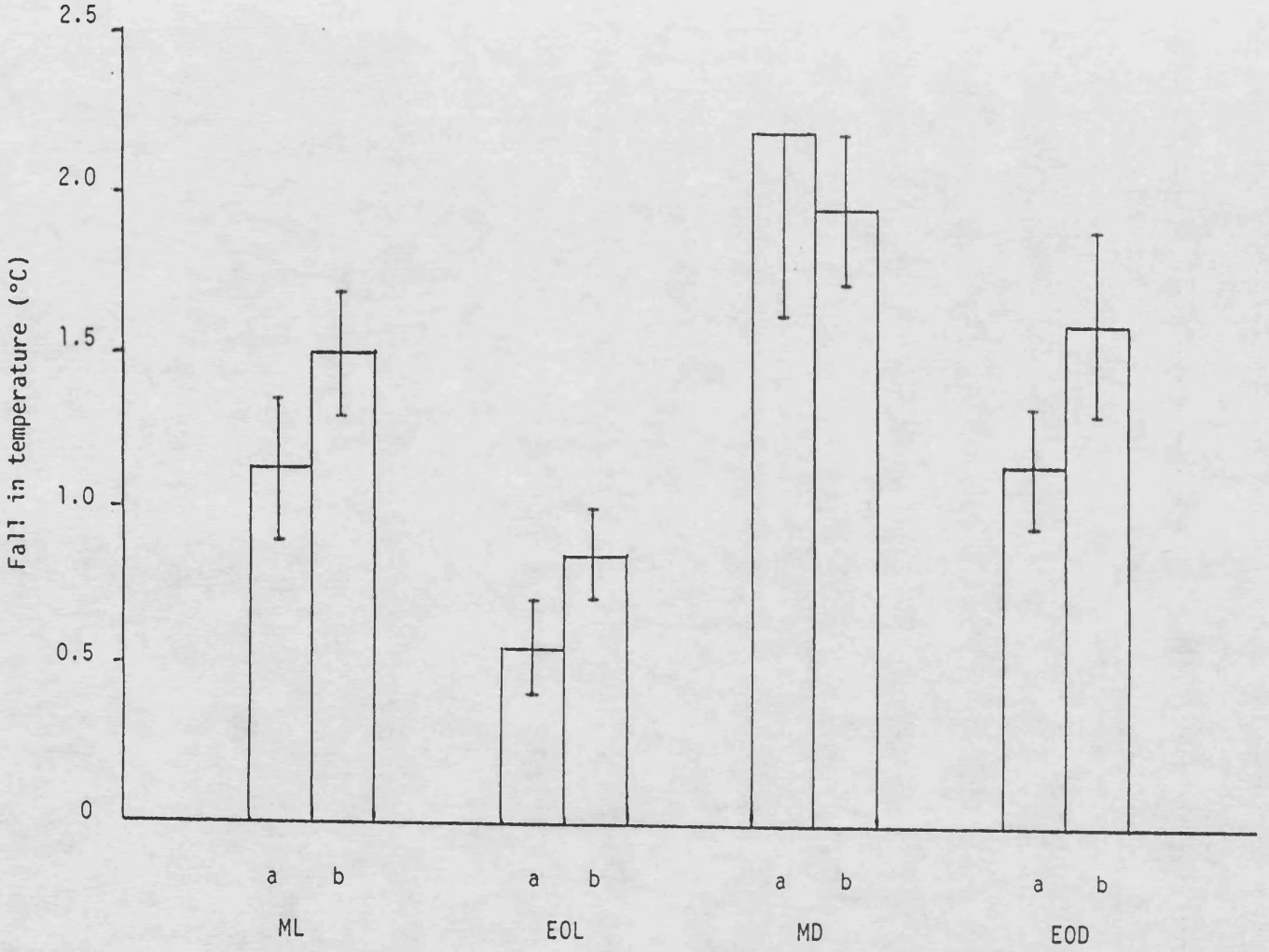


Fig. 3.6. Effect of melatonin on 8-OHDPAT-induced hypothermia in mice. (n=6, mean ± s.e.m.).



Key:

a = 8-OHDPAT (0.5mg/kg): saline

b = 8-OHDPAT (0.5mg/kg): melatonin (1mg/kg)

ML = mid-light

EOL = end-of-light

MD = mid-dark

EOD = end-of-dark

Fig. 3.7. Dose-response curve showing the induction of hyperphagia by 8-OHDPAT in rats. (n=8, mean ± s.e.m.).

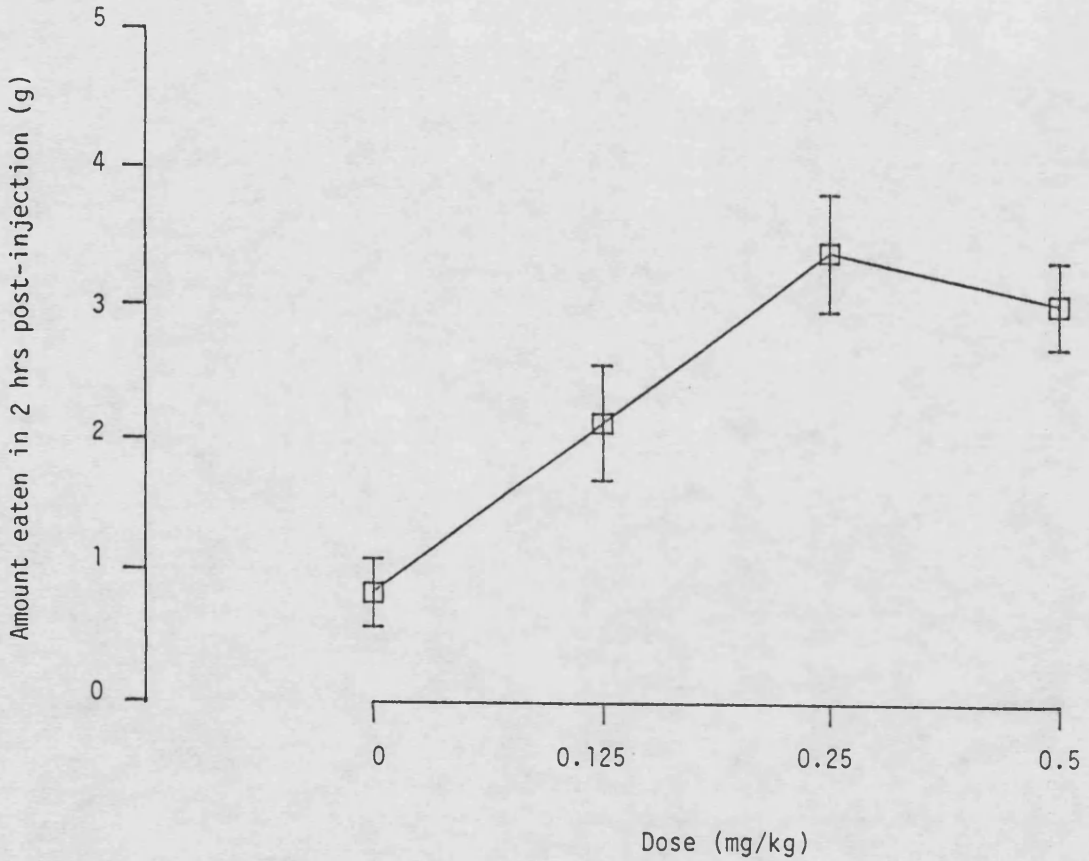
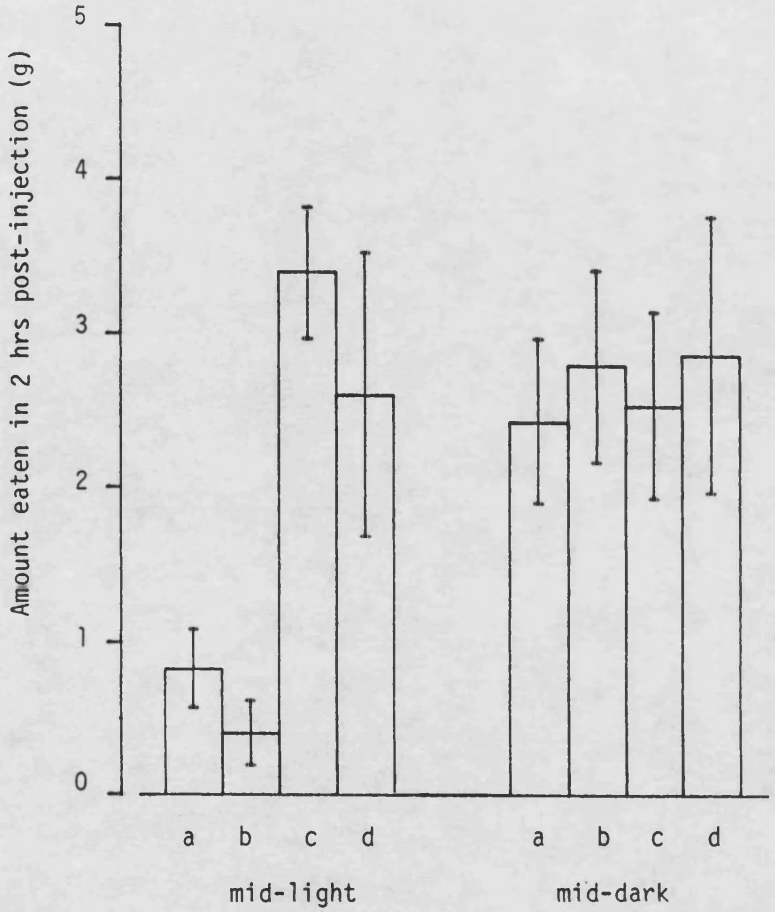


Fig. 3.8. Effect of melatonin on 8-OHDPAT-induced hyperphagia at mid-light and mid-dark in rats (n=6-8, mean ± s.e.m.).



Key:

a = vehicle : vehicle

b = vehicle : melatonin (1mg/kg)

c = 8-OHDPAT: (0.25mg/kg) : vehicle

d = 8-OHDPAT: (0.25mg/kg) : melatonin (1mg/kg)

Fig. 3.9. Dose-response curve showing the induction of hyperactivity by RU24969 in mice. (n=8, mean ± s.e.m.).

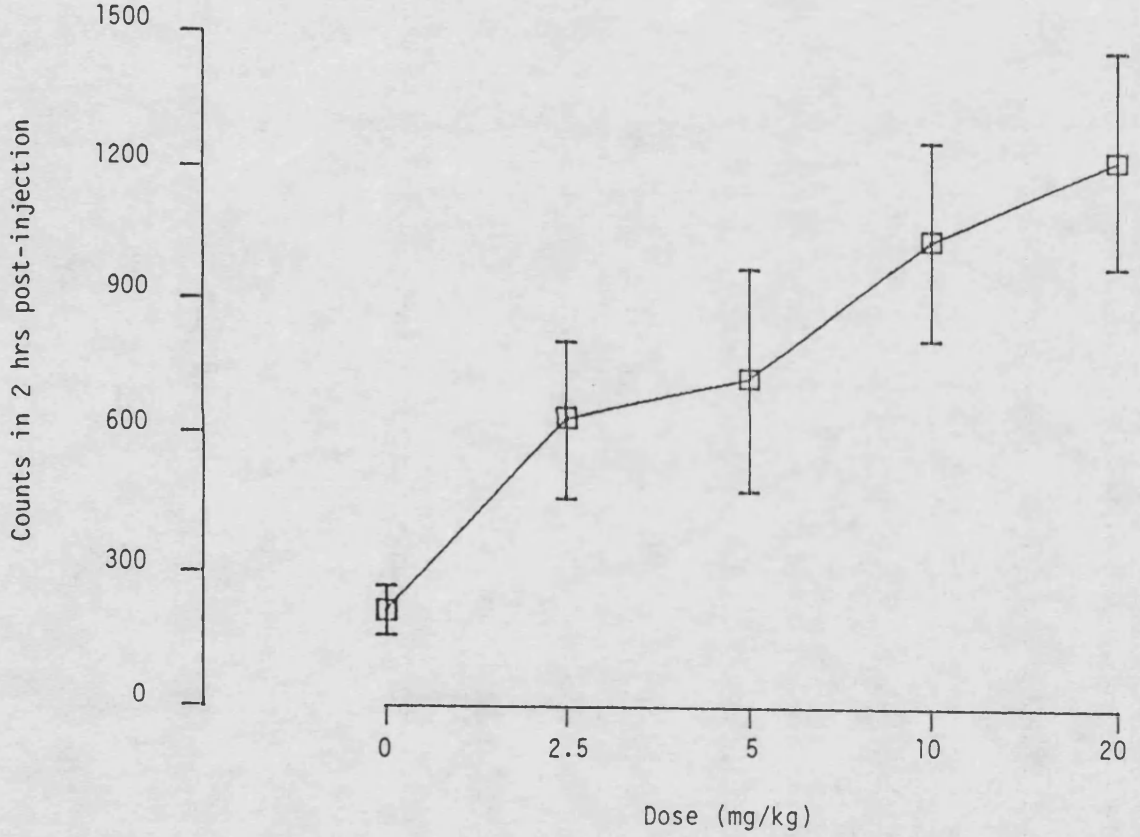
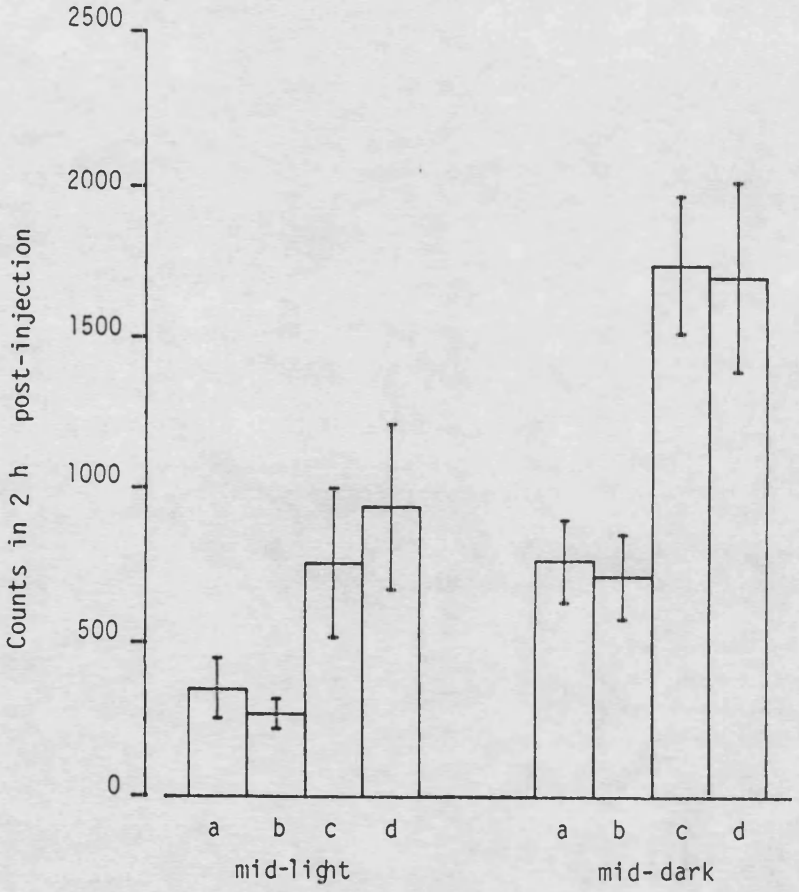


Fig. 3.10. Effect of melatonin on RU24969-induced hyperactivity at mid-light and mid-dark in mice. (n=8, mean ± s.e.m.).



Key:

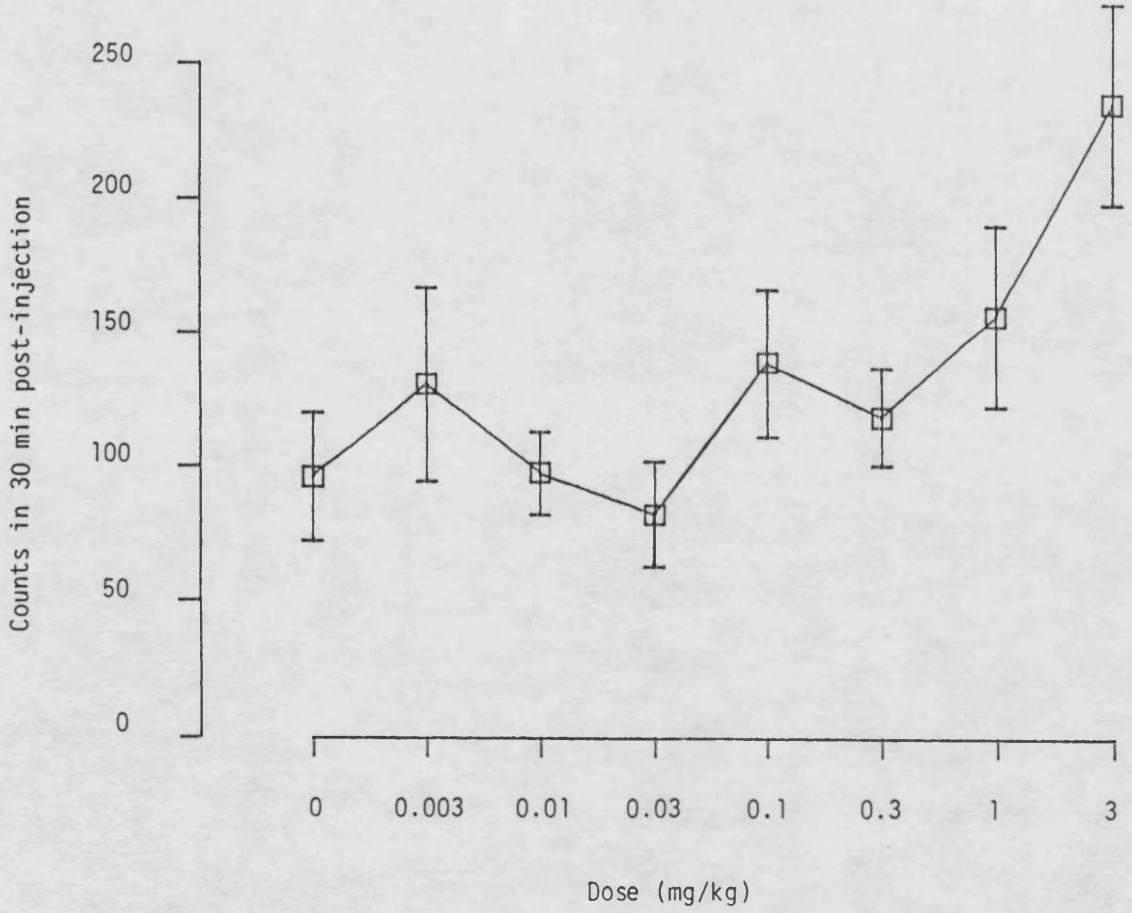
a = saline : saline

b = saline : melatonin (1mg/kg)

c = RU24969 (5mg/kg) : saline

d = RU24969 (5mg/kg) : melatonin (1mg/kg)

Fig. 3.11. Effect of low-dose RU24969 on locomotor activity in mice. (n=6-8, mean ± s.e.m.).



3.4. Discussion

The singular ineffectiveness of melatonin in this series of experiments was surprising. It does not rule out, however, any possibility of an interaction between these two systems since the results may simply reflect that the sites of interaction are physiologically and pharmacologically distinct from the serotonergic sites that are responsible for 5-HT-mediated behaviour. The doses of melatonin used in this study could be considered low but there is evidence to suggest that melatonin is a potent molecule (e.g. see Cassone et al., 1986a; Reiter, 1983b, and references cited therein) and, due to its lipophilic nature and the free transport of plasma albumin-bound melatonin across the blood brain barrier, can rapidly penetrate the brain following i.p. injection (Reppert et al., 1979; Sugden, 1980; Pardridge and Mietus, 1981). Since this work was carried out it has been shown that similar doses of melatonin can affect 5-HT_2 receptor mediated sleep-wakefulness regulation in the rat (Dugovic et al., 1989). Involvement of the serotonergic system in sleep is well established and has been implicated in both the initiation and maintenance of slow-wave sleep and paradoxical sleep (REM) (Morgane and Stern, 1972). Holmes and Sugden (1982) reported an increase in these components of sleep following a melatonin dose of 10mg/kg. The work of Dugovic and co-workers indicate the potential involvement of 5-HT_2 receptors in regulating slow-wave sleep in the rat since ritanserin, a 5-HT_2 selective antagonist, was found to reverse the effect of the 5-HT_2 agonist DOM on deep slow wave sleep (Dugovic and Wauquier, 1987; Dugovic and Wauquier, 1988; Dugovic et al., 1989a; Dugovic et al., 1989b). An observation that the effectiveness of ritanserin depended on the time of administration during the LD cycle prompted the investigation as to

whether it was linked to melatonin. Using a dose of 1mg/kg Dugovic et al. (1989a) reported that although melatonin failed to influence any sleep stage when given alone it antagonized the opposite effects of both ritanserin and DOM on deep slow-wave sleep and paradoxical sleep. The mechanism behind this interaction is not understood though Dugovic concludes that melatonin modulates 5-HT2 receptor sensitivity possibly through a mechanism involving arginine vasotocin (AVT). The lack of affinity of melatonin for 5-HT2 receptors effectively eliminates "classical" pharmacological explanations that melatonin might be a partial agonist at this site (Dugovic et al., 1989b; Pickering and Niles, 1990). Activation of the same 5-HT receptors located in the brain stem are thought responsible for controlling 5-HT-mediated headtwitches (Bedard and Pycock, 1977), but the failure of melatonin to influence their manifestation would suggest that it exerts no influence on these receptors. An investigation into the effects of melatonin treatment on 5-HT2 binding sites and their putative high and low-affinity components is required. It should be remembered that "slow-wave sleep" and "paradoxical sleep" are classified according to the pattern of electrical activity in the brain and are therefore the products of the electrical activity of many interacting neurotransmitter systems. Thus melatonin may simply act via a compensatory mechanism unconnected with the serotonergic system to restore the balance disturbed by abnormal serotonergic activity. It would be of interest to study the effects of putative melatonin antagonists in this model, since it would give an indication as to whether melatonin acts through its own receptor or exerts a generalized hypnotic action. 5-HT-agonist induced headtwitches is more specific as a model for investigating a potential interaction

between melatonin and 5-HT. Although a variety of compounds can induce head shaking behaviour, e.g. carbachol, TRH (thyrotropin-releasing hormone) and opiates (Wei et al., 1977; Prange et al., 1974; Handley and Singh, et al., 1986) these do not appear to involve a serotonergic mechanism (Bedard and Pycock, 1977; Drust and Connor, 1983).

The effect of prolonged melatonin treatment (either as a single bolus daily dose or continuous infusion) in 5-HT-mediated behavioural models merits further investigation. Despite the lack of effect of chronic melatonin therapy on 5-HTP-induced headtwitches it does not eliminate the involvement of other 5-HT receptor subtypes - (although it should be mentioned that diminution of the 5-HT-syndrome induced in conjunction with the headtwitches by 5-HTP was not apparent during the course of the experiment in comparison to acute melatonin treatment). Much attention has been paid to the relationship between melatonin, depression and antidepressants in recent years notwithstanding the established, if little understood, role of the serotonergic system in major affective disorder. Acute treatment with tricyclic antidepressants increasesplasma melatonin levels as a result of increasing the availability of noradrenaline to stimulate beta-adrenoceptors in the pineal gland by blocking noradrenaline reuptake (Parfitt and Klein, 1977; Burns and Brown, 1984; Franey, et al., 1986). Chronic antidepressant treatment elicits adaptive changes at adrenoceptors, the most consistent being a reduction in either the number of beta-adrenoceptors or their coupling to the cAMP second messenger system (Sulser et al., 1984; Moyer et al., 1979; Heydorn et al., 1982). This leads to a concomitant decrease in pineal and plasma melatonin levels (Heydorn et al., 1982; Friedman et al., 1984). Reduced levels of circulating

melatonin in depressed patients has been repeatedly observed as a feature of depression (Lewy et al., 1979; Wirz-Justice and Arendt, 1979; Wetterberg, 1983; Steiner and Brown, 1985) and may be the result of reduced central noradrenergic tone associated with depressive illness and perhaps deficiency in the melatonin precursor 5-HT (Heninger et al., 1984; Arendt, 1989). Conversely, Thompson et al. (1988) have recently reported no differences in the timing or amplitude of melatonin secretion between depressives and controls which suggests that melatonin is unlikely to be involved in the aetiology of depression. Moreover, reduced melatonin production caused by chronic antidepressant treatment does not affect their clinical efficacy. However, melatonin does demonstrate potential for use as a diagnostic aid in certain psychiatric conditions (Miles and Thomas, 1988; Arendt, 1989). It is generally recognized that an important contributory factor in the action of certain antidepressants is their ability to increase central serotonergic tone (Sulser et al., 1978; Sulser, 1983) either through reduction of 5-HT catabolism or inhibition of reuptake mechanisms. Antidepressants have mixed actions on 5-HT receptors; tricyclic antidepressants (TCAs; e.g. amitryptiline, nortryptiline) tend to inhibit headtwitches (Ogren et al., 1979) whereas MAOIs (monoamine oxidase inhibitors) and 5-HT reuptake inhibitors (e.g. fluoxetine) potentiate head-shaking behaviour (Matthews and Smith, 1980). Chronic administration of antidepressants, however, lead to inhibition of headshaking behaviour if tested during treatment - a response thought to result from the down-regulation of 5-HT_2 receptors (Lucki and Frazer, 1985; Peroutka and Snyder, 1980) (it should be noted though that prolonged antidepressant therapy down-regulates alpha_2 adrenoceptors too and these receptors are also

thought to be involved in the mediation of headtwitches (Glennon and Lucki, 1988)). The 5-HT syndrome induced by 5-HT agonists is reduced following chronic MAOI therapy, an action thought to be related to their ability to down-regulate 5-HT$_1$ receptors in the brain stem or spinal cord (Lucki and Frazer, 1982). This leads on to the observations that repeated administration of antidepressants inhibits the hypothermic response to 8-OHDPAT (Goodwin et al., 1985), and chronic MAOI treatment (but not TCAs) blocks the activity-suppressant effects of mCPP and TFMPP - putative 5-HT$_{1B}$/5-HT$_{1C}$ selective agonists (Lucki and Frazer, 1985). Furthermore, Gaffori and Van Ree (1985) demonstrated that behavioural changes induced by melatonin injected into the rat nucleus accumbens were reversed by acute treatment with 5-HT and antidepressants but not by dopamine antagonists. It would therefore be justified to investigate further the relationship between chronic melatonin therapy and the serotonergic system to establish whether their mutual involvement with depression and antidepressants is active or coincidental.

The absence of any effect of melatonin on the rectal temperature of mice ruled out the possibility that melatonin might accentuate or reverse 8-OHDPAT-induced hypothermia by an indirect mechanism. The concept that melatonin fails to interact with 5-HT due to its inability to reach the appropriate serotonergic site as mentioned above is also less tenable as an explanation for its lack of influence on 8-OHDPAT-induced hypothermia. This is because the hypothalamus, generally recognized as the thermoregulatory centre of the body, is likely to be the ultimate site of action for 5-HT, since hypothalamic depletion of 5-HT by 5,7-DHT or pCPA attenuates the hypothermic response to 8-OHDPAT (Goodwin et al., 1985). It is thought that 8-OHDPAT activates the 5-HT$_{1A}$ somatodendritic receptor

located on the 5-HT cell bodies of the raphe nuclei leading to the inhibition of 5-HT synthesis which causes the hypothermic response (Goodwin et al., 1985; Goodwin et al., 1987). Current evidence indicates the hypothalamus as being the most likely major CNS site of action for the physiological effects of melatonin (Armstrong, 1989; Morgan and Williams, 1989). The same subclass of 5-HT_1 receptors are thought to be involved in the hyperphagic effect of 8-OHDPAT. Interestingly, though, food-deprived animals display hypophagia after 8-OHDPAT administration (Bendotti and Samanin, 1987). There are several examples of irregularities recorded in the literature concerning monoamine involvement of thermoregulatory control and feeding behaviour (e.g. Bligh et al., 1971; Milton, 1978). More selective 5-HT antagonists are required to help resolve the situation, although the dichotomous nature of the action of 8-OHDPAT may well be explained by internal adjustment of receptor sensitivity resulting from the change in food availability. Inter-species variation in responsiveness to manipulation of the serotonergic system may be due to slight anatomical differences in the arrangement of neuronal pathways, or the ease of penetration of the drug to the active site, or the temperature of the external environment which in turn influences the sensitivity of the organism to drug treatment (Goodwin et al., 1985). Gudelsky et al. (1986) has provided evidence for opposing roles of 5-HT_{1A} and 5-HT_2 receptors in temperature regulation whereby activation of the 5-HT_2 receptor subtype elicits hyperthermia. This proposal was based on the observation that 5-HT_2 antagonists countered the hyperthermia induced by 5-HT agonists, e.g. quipazine, 5MeODMT (high doses), 6-chloro-2-(1-piperazinyl)- pyrazine (MK-212). Additionally, prior to their recognition as reasonably selective 5-HT_2 agonists, DOM and

DOB were known to cause hyperthermia in rodents (Aldous et al., 1974). A more appropriate model, therefore, for studying potential 5-HT_2 receptor/melatonin interactions might be 5-HT_2 receptor-mediated hyperthermia rather than headtwitches due to the common locality of action - the hypothalamus.

8-OHDPAT-induced feeding was also considered to be a suitable model to assess the effect of melatonin on the activation of 5-HT_{1A} receptors. Melatonin alone did not alter the amount of food eaten in comparison to control animals. The inability of melatonin to affect 8-OHDPAT-induced hyperphagia does not necessarily suggest that it plays no role in regulating the serotonergic aspect of feeding behaviour. Activation of the serotonergic system using, for example, 5-HT releasing agents such as fenfluramine, was previously thought to cause anorexia rather than hyperphagia (Blundell, 1977). It has also recently been shown that putative 5-HT_{1B} and 5-HT_{1C} selective agonists induce anorexia - an action apparently effected through the paraventricular nuclus of the hypothalamus (Hutson et al., 1988). Kennett and Curzon (1988a, 1988b, 1988c) published a number of reports which extended the results of Hutson et al. (1988) to evince a complex relationship between 5-HT_1 receptor subtypes and the serotonergic input into the regulation of feeding behaviour. Their effect would appear to be due to a direct action on the hypothalamus rather than the raphe nuclei which provide serotonergic input to this region (Hutson et al., 1988). An extended study would be desirable to investigate whether melatonin interferes with the involvement of these other 5-HT_1 receptor subtypes.

The hypnotic properties of melatonin would lead one to expect that hyperactivity induced by RU24969 might be attenuated by

melatonin treatment. Its lack of effect suggests that melatonin exerts no influence on the expression of 5-HT$_{1B}$-mediated behaviour. This conclusion, however, may not be considered absolute for three reasons. Firstly, as discussed above, the effect of chronic melatonin treatment was not investigated in this paradigm. Secondly, it has recently been shown that RU24969 exhibits nanomolar affinity for melatonin binding sites in the hypothalamus (Pickering and Niles, 1990). This is probably due to the common indole nucleus shared by the compounds, though it is not yet known in what capacity RU24969 acts, if indeed the melatonin binding site is a functional receptor. Melatonin has negligible affinity for the 5-HT$_{1B}$ receptor. Thirdly, RU24969-induced hyperactivity is not an ideal model for 5-HT$_{1B}$ receptor activation (e.g. Tricklebank, 1985) since centrally-acting beta-adrenoceptor antagonists (e.g. (-)-propranolol), used here in their capacity as 5-HT$_{1A}$/5-HT$_{1B}$ receptor antagonists, fail to consistently block its appearance (Green et al., 1984; Tricklebank, 1984). Indeed, it is now considered that RU24969-induced anorexia is a more appropriate indicator of 5-HT$_{1B}$ receptor activation (Kennett et al., 1987; Kennett and Curzon, 1988b), an effect thought to be mediated through a post-synaptic site mechanism due to its persistence after pCPA treatment (Kennett et al., 1987). Additionally, hypoactivity and anorexia induced by the putative 5-HT$_{1B}$ and 5-HT$_{1C}$ selective agonists (TFMPP and mCPP) appear to result from 5-HT$_{1C}$ receptor activation. This has been presumed because (-)-propranolol fails to inhibit mCPP or TFMPP-induced hypoactivity (Kennett and Curzon, 1988). Furthermore, metergoline and mianserin, recognized 5-HT antagonists with high affinity for 5-HT$_{1C}$ receptors (Hoyer et al., 1985; Sanders-Bush and Breeding, 1988), reversed this behaviour and

inhibited mCPP-induced hypophagia (Kennett and Curzon, 1988). 5-HT$_{1c}$ mediation of hypoactivity would also explain why RU24969 failed to produce this behaviour in rats when tested.

It was thought that circadian variation in the sensitivity of either the serotonergic system or putative melatonin receptors might determine the extent of an interaction between melatonin and 5-HT. Several reports indicate that the absence of melatonin in the plasma during the daylight hours might render the body most receptive to melatonin treatment at the end of the light phase (e.g. Reiter, 1983b; Redman et al., 1983; Armstrong, 1989). (It should be noted, however, that the literature concerning the quotidian variation in sensitivity towards melatonin is inconsistent. Zisapel et al. (1988) have reported that melatonin binding in the hypothalamus is maximal at the end of the light phase. Conversely, the work of Laitinen et al. (1989) suggests that sensitivity, as measured by receptor affinity, reaches a zenith at the end of the dark phase). Despite conducting the temperature and headtwitch experiments at four time points spaced evenly apart along the 24 h LD cycle there was no observation of an interaction. Feeding and activity experiments, conducted at ML and MD also failed to demonstrate an interaction, though a late afternoon experiment might have been a more appropriate observation time in these instances. These results do, however, demonstrate a curious feature of 8-OHDPAT-induced hyperphagia. The amount of food consumed by 8-OHDPAT-treated animals at MD does not differ from control animals, unlike those treated at ML. Similar results have recently been reported by Sleight et al. (1988) who found that animals exhibited hypophagia if given 8-OHDPAT during their dark phase. This suggests that the 5-HT receptors involved in the stimulation of feeding are

maximally stimulated at night, which would concur with the nocturnal feeding habits of these rodents. The hypophagia detected by Sleight et al. (1988) is attributed to an inhibitory action mediated via a peripheral mechanism. Conversely, the hyperactivity induced by RU24969, at the doses employed, does not suggest that the receptors responsible for mediating this activity are maximally stimulated during the dark phase despite this being their normally active phase. This, in turn, suggests that hyperactivity is induced via a mechanism not shared by the system responsible for initiating night-time associated activity.

In conclusion, it can be said that melatonin had no effect on the behavioural models of 5-HT mediated behaviour used in this study. However, recent work by Dugovic and co-workers (Dugovic and Wauquier, 1988; Dugovic et al., 1989a; Dugovic et al., 1989b) on the sleep-wake cycle suggest that an indirect interaction may exist, although its precise nature is not yet known.

CHAPTER 4.

DRUG DISCRIMINATION

4. DRUG DISCRIMINATION

4.1. Introduction

Behaviour expressed by an animal in normal circumstances can be described as being "emitted" or "elicited" according to whether it has been induced by a stimulus or not. Emitted behaviour is said to occur when there is no apparent, simple to describe, stimulus. Its analysis prior to artificial modification is within the realms of the ethologist, observing and recording each element of the expressed behaviour, e.g. grooming, running, headtwitches, posture etc. Elicited behaviour is that element which can be seen to be caused by a readily definable stimulus, the classic example being the retraction of a limb when placed on a hot surface. Both types of behaviour can be suitably modified by experimenters such that a discrete "package" of behavioural elements can be reliably repeated. This process is known as conditioning. This form of simplifying behavioural parameters into discrete measureable, automated responses has had a major impact on the science of psychology. Exploitation of these conditioning experiments in turn has made an equally profound impact on the study of the influence of drugs on behaviour - psychopharmacology. Two types of conditioning exist: classical (also known as "Pavlovian", respondent, or Type 1) and Operant (Instrumental or Type 2). Analysing the influence of drugs on conditioned behaviour has permitted a quantitative evaluation of an essentially qualitative behavioural characteristic. For example, the well known pairing of a bell ringing at the presentation of food leads to salivation in an animal after only the bell has been rung. The food was the initial unconditioned stimulus which causes the unconditioned response of salivation. However, once

pairing has been established, the sound of the bell, the conditioned stimulus, induces the response (now known as a conditioned response). Thus an elicitted behaviour has been conditioned by way of the classical technique. Emitted behaviours are conditioned using the operant technique. That is if a certain behaviour expressed by an animal results in a pleasurable experience then the probability of the animal repeating that element is increased (reinforcement). If however, it results in a painful experience then the probability of it happening again is reduced (punishment). Anthropomorphically it can be seen that there is a blurred edge to the differentiation of what constitutes an operant technique of conditioning and what is a classic technique. For example the removal of a limb from a hot surface is an operantly-defined act if one considers the pleasure experienced at the relief of pain. Philosophical arguments on the complexity of whether an animal is operantly or classically conditioned are beyond the scope of this thesis. Suffice to say that although this chapter represents work that is an example of psychopharmacological exploitation of operant conditioning, an element of classical conditioning is present.

The T-maze has become synonomous with discrimination. Under the influence of an exteroceptive or interoceptive stimulus an animal has to make a choice at the junction of the "T" whether it is more advantageous to travel along the left arm or the right. The emitted behaviour, that of running, is capitalized upon and encouraged by the experimenter. The animal is required to find the "reinforcement" area. To encourage the search an animal is usually provided with a recognizable "motivation factor", e.g. escaping a mild electric shock or, as a result of "deprivation", a "satisfier". Thus the term "reinforcement area" has been chosen to describe a

site whereby an animal can, for example, acquire food or water or escape a shock. The introduction of the Skinner box into the field of drug discrimination by Barry (1968) added a further dimension to its study. Workers could now assimilate a much greater wealth of information as a result of analyzing the effects of a test drug on response latency and response rate in conjunction with its discriminability. The emitted behaviour in this case is the depression of a lever which brings either relief from deprivation or relief from shock. Although it is possible to study discriminative behaviour with just one lever in the operating chamber, the standard practice is to use two. For example the illumination of a coloured light (exteroceptive cue) might be indicative of reinforcement being available if the left lever is pressed whereas if the light is off the right lever must be pressed. This principle is the same for interoceptive cues where if an animal has been given either a drug or saline (or in some instances another type of drug) - the drug must evoke some form of identifiable "state" to the animal to distinguish it from the "control", undrugged, "state". Despite a patchwork of research into various aspects of discrimination since the science of psychology and even philosophy, began, pharmacological interest was minimal until the 1960's when Overton commenced a series of investigations into what was described as State Dependent Learning (SDL) (Overton, 1961). SDL is said to occur when an animal, trained to perform a certain task under the influence of a specific condition (usually an internal "state" evoked by the administration of a centrally-acting drug), is unable to repeat the task in the absence of that condition.

SDL and drug discrimination are further examples of overlapping techniques/ disciplines, though it can be argued that

the latter is a subset, a more refined element, of State Dependent Learning. They tend to be separated more on methodological grounds than by discrepancies in descriptions of the internal milieu by which an animal must discriminate from an opposing condition. This is because both depend on the animal behaving in one manner under the influence of the drugged condition and in another mutually exclusive manner in the undrugged state. The use of one lever in the Skinnerian method of drug discrimination exemplifies the fragile delineation between it and SDL. In this method a group of animals are trained to press the lever in the drugged state (often referred to as S^+), but not to respond when given saline (often referred to as S^-). The procedure is reversed for a second group. Hence the preponderance of dual lever responding in drug discrimination studies. Colpaert (1977) discusses the evidence pertaining to differentiating between SDL and drug discrimination. Part of the argument resides with the concept that for a drug to induce SDL, it must create a "state" within the animal via a central mechanism, i.e. the drug must act centrally. Drug discrimination learning, it is reasoned, does not require this criteria. Thus a peripherally acting drug can provide a discriminative cue, e.g. arthritic rats can distinguish between saline and aspirin. However this situation could be considered as a type of "reversed discrimination" since the disease arthritis has established a "state" within the organism which has become the norm (presumably comprised of an awareness of reduced mobility and consequential pain). The removal of this state therefore establishes a novel awareness - the "drugged" state, that is to say that indirectly the drug has acted centrally to change the internal perceptual environment of the animal. Hence the ability of a non-narcotic agent to provide a discriminable stimulus that is

dependent upon the internal state of the organism. Thus, although it is widely accepted that drug discrimination can occasionally be expressed by peripherally acting drugs, their discriminability is dependent upon some perceived change in the internal environment of the animal regardless of the manner by which it is brought about. This statement is substantiated by the fact that there are very few reports of a drug incapable of penetrating the blood-brain barrier acting as a discriminative cue. The vast majority of reports describe the discriminative characteristics of centrally acting drugs. The ability of these drugs to elicit an identifiable internal state upon an organism varies and is related to the pharmacological potency of the compound under study. The doses involved, however, are generally lower than those required to elicit an observable change in the unconditioned behaviour of a subject animal.

The use of drug discrimination as a research tool of the behavioural pharmacologist has rapidly increased since the mid 1970's and become a standard technique for the screening of potential psychoactive drugs within the pharmaceutical industry. Drugs of similar action are indistinguishable from the original training compound by the animal, i.e. its response to receiving the novel drug is the same as if it had been given the training drug. This is the basis of generalization studies (also known as transfer studies). Antagonism studies investigate the ability of a compound to interfere with the expression of the training drug's cue in much the same way as conventional antagonism studies, and therefore the animal is expected to make the response appropriate to its undrugged state. Indeed the drug discrimination procedure has widespread use in industry as a means of classifying novel compounds with respect to existing psychoactive drugs.

Dose response curves can be constructed both with the test and training compounds. The gradient of the curve derived from the training drug reveals the dose range over which the "drugged state" of the animal can act as a cue for a drug-appropriate response. This range bears no relationship to the dose range of a drug required to influence unconditioned behaviour and is usually below the minimal dose required to influence unconditioned behaviour. Dose response curves generated by test drugs reveal the nature of their own influence on the inner state of the animal. Drugs of a different pharmacological class to the training compound tend to induce a saline-appropriate response. It is possible to achieve partial generalization with a compound - an effect under an increasing amount of investigation (Colpaert, 1988, Stolerman et al., 1987) which in turn is suggestive of partial agonist activity on the receptor(s) responsible for cue generation. However, drug discrimination can be as "fickle" as standard pharmacology, producing a complete generalization at one dose and partial generalization at a higher one. This is often the result of behavioural disruption incapacitating a percentage of the animals, e.g. the action of 5MeODMT on L-5HTP trained animals. A more interesting example of the potential for varying generalization characteristics has been described by Stolerman et al. (1984). The two groups of rats used, one trained to discriminate 0.1mg/kg nicotine from saline and the other trained to differentiate between 0.4mg/kg nicotine and saline, expressed dissimilar stimulus transfer characteristics for anabasine, the lower dose nicotine group reaching 100% generalization at the appropriate anabasine dose, while the other could achieve no more than 50%. This suggests that the nature of the cue itself generated by a test compound can be

dose dependent. It would have been interesting to find out whether a third group of animals could differentiate between the lower and upper doses of nicotine employed. Antagonists produce similar dose-response curves directed towards inhibiting the expression of the cue and may also exhibit partial antagonism. As with any classical test for a competitive antagonist the dose response curve (in this case dose-generalization curve) is shifted to the right (see Colpaert and Janssen, 1983).

The serotonergic system has long been associated with drug discrimination, initially through the study of the discriminative properties of the hallucinogen LSD, and provides a good example of the contribution that drug discrimination can make towards understanding the functional aspects of receptors. Hirschhorn and Winter demonstrated the similarity of cues induced by LSD and mescaline as early as 1971. Since then various workers have made progress towards the goal of establishing its origin. The transferance of the mescaline-induced cue to non-hallucinogenic compounds nullified theories that such a characteristic was common only to hallucinogens (Kuhn, 1977). This would agree with the view that the ability to discriminate between a drugged and non-drugged state depends on a conscious awareness by the animal of that change in state. If, for example, discrimination only depended on the level of consciousness as perceived by an animal when given a CNS depressant then the demonstration of specificity in discrimination studies would be rare. That is, sedation, would act as a cue whether caused by a barbiturate, benzodiazepine or centrally acting antihistamine - the route to discrimination may be different as a result of action on different systems, but the destination is the same. However, the use of different drug tools allow the specificity

of a compound, with respect to its cue-generating properties, to be determined by antagonism studies (e.g. see Young and Dewey, 1982). The discrimination between mescaline and LSD suggests that despite acting on apparently different neurotransmitter systems the internal manifestation of the cue is essentially the same, which in turn implies the existence of a common pathway for the cue at some point from its neuronal origins to cognizance, and that this pathway is shared by non-hallucinogenic (but still psychoactive) drugs. In this, drug discrimination can be viewed as a pharmacological effect that requires as much thought and caution as any other *in vivo* or *in vitro* technique. This concept is discussed further in detail by Rosecrans et al. (1978).

LSD is now recognized to act as a central 5-HT agonist, despite initial evidence to the contrary based on the findings of Gaddum and Piccarelli (1957) who demonstrated that it antagonised the action of 5-HT on intestinal smooth muscle. Aghajanian et al. (1970) found that LSD, mimicking the effect of 5-HT, increased the firing rate of those brainstem neurons that received afferents from 5-HT-containing cells of the raphe nucleus. Additionally, LSD, like 5-HT, inhibits the firing rate of 5-HT neurons in the raphe. Receptor binding studies have shown LSD to be effective in displacing 5-HT from both 5-HT_1 and 5-HT_2 receptors (Leysen et al., 1981; Peroutka and Snyder, 1979). This contrasted with certain behavioural studies concerning the activity of LSD, most notably drug discrimination, which suggested that the 5-HT_2 receptor was the most influential with regards to the LSD generated cue. Pirenperone, a 5-HT_2 selective antagonist, was shown by Colpaert et al. (1982) to inhibit the the LSD cue followed by ritanserin, another highly selective 5-HT_2 antagonist, (Colpaert et al. 1985).

In addition to this, the cue properties of DOM and quipazine, both of which generalize to LSD (Kuhn et al., 1978; White and Appel, 1982) were antagonised by pirenperone (Friedman et al. 1984; Glennon et al. 1983). More recently, Cunningham and Appel (1987) have investigated the LSD cue with reference to 5-HT$_1$ selective agonists. The 5-HT$_{1A}$ receptor agonist 8-OHDPAT, the mixed 5-HT$_{1A}$/5-HT$_{1B}$ agonist RU24969 and the mixed 5-HT$_{1B}$/5-HT$_{1C}$ agonists TFMPP and mCPP did not induce LSD-appropriate responding. Interestingly, the non-selective 5-HT-receptor agonist/hallucinogen, 5MeODMT, and the purported 5-HT$_2$ agonist DOM, also a hallucinogen, have been shown to express discriminative cues indistinguishable from LSD regardless of the training drug (Glennon et al. 1979; 1983a; 1983b; 1984b). Standard behavioural studies have shown 5MeODMT to be a potent promoter of the so-called serotonin syndrome (Jacobs, 1976) which has components induced by 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_2$ receptor activation (see chapter 3, this thesis). Identifying the focal point of the discriminative cue has proved difficult. The LSD cue generalizes only to agents that have been recognized to have 5-HT$_2$ receptor agonist properties. Neither TFMPP nor 8-OHDPAT share stimulus transfer characteristics with LSD (Cunningham and Appel, 1987; Tricklebank et al. 1987) despite displaying partial (in the case of 8-OHDPAT) or full generalization with 5MeODMT. Thus, the cue generated by 5MeODMT must have both 5-HT$_1$ and 5-HT$_2$ receptor involvement with the 5-HT$_2$ receptor mediated cue taking precedence over the 5-HT$_1$ otherwise LSD would display partial rather than full transfer characteristics. Circumstantial evidence for this view is given by Stolerman (1987) who used a mixture of nicotine and midazolam to generate a discriminative cue. Once trained, rats displayed partial generalization to midazolam and nicotine if given

separately and partial antagonism if given mecamylamine (nicotine antagonist) or Ro 15-1788 (benzodiazepine antagonist) separately. Complete inhibition of the midazolam/nicotine cue was achieved if a mixture of the antagonists was administered.

The more selective 5-HT receptor ligands have themselves been subjected to drug discrimination analysis but have yielded contradictory data. Tricklebank et al. (1987) investigated the origin of the cue generated by 8-OHDPAT and concluded that activation of the 5-HT$_{1A}$ receptor appeared to be the sole source. Similarly, the 5-HT$_{1A}$ receptor has been implicated in the derivation of the cue induced by the novel non-benzodiazepine anxiolytic buspirone (Mansbach and Barrett, 1987). TFMPP serves also as a discriminative stimulus (Glennon et al. 1984) as determined by its action on 5-HT$_{1B}$, and possibly 5-HT$_{1C}$, receptors (Cunningham and Appel, 1986; McKenney and Glennon, 1986; Glennon et al., 1988). Further work on TFMPP by Glennon et al. (1988) in the field of drug discrimination has questioned the 5-HT$_{1A}$/5-HT$_{1B}$ antagonism properties of propranolol and pindolol as a result of their stimulus generalization to TFMPP. Glennon et al. (1988) suggested that centrally acting non-selective beta-adrenoceptor antagonists may possess agonist characteristics at certain populations of 5-HT receptors, an idea indirectly supported by the work of Cunningham et al. (1987) who failed to block 8-OHDPAT cue with propranolol. This deserves further investigation since it may be that TFMPP (the training drug used in the study by Glennon et al. (1988)) is displaying partial agonist characteristics. This contention is supported by the results of Tricklebank et al. (1987) who were able to demonstrate dose dependent antagonism of the 8-OHDPAT-induced cue by pindolol.

L-5HTP was first used successfully as a training drug by Barrett et al. (1982), who suggested that its use in operant behaviour prior to this was restricted due to disruptive behavioural effects attributed to a peripherally-mediated action. By employing a decarboxylase inhibitor, RO4-4602, that was unable to penetrate the blood-brain barrier, Barrett was able to demonstrate that the cue involved "centrally formed decarboxylated products". This was further supported in the same paper by showing that the inhibition of brain L-amino acid decarboxylase with NSD-1015 completely inhibited the generation of the cue. The classical 5-HT antagonists, cyproheptadine, metergoline, methiothepin and methysergide failed to inhibit L-5HTP-appropriate responding and it was suggested that, along with evidence presented in a subsequent publication (Friedman et al., 1983), the L-5HTP cue was mediated by a unique 5-HT receptor. Cunningham et al. (1985) reported that L-5HTP induced a complex cue derived from both 5-HT_1 and 5-HT_2 receptor activation as defined by Peroutka et al. (1981). The basis for this proposal was the inhibition of LSD substitution by the 5-HT_2-selective antagonist ketanserin in L-5HTP trained rats. The development of more selective 5-HT_1 agonists enabled Moser and Redfern (1985b) to assign responsibility for the cue to the 5-HT_1 receptor since attempts to inhibit it with various 5-HT_2 antagonists failed. Additionally, TFMPP, RU24969 and 5MeODMT fully substituted for L-5HTP while 8-OHDPAT expressed partial generalization. This result led Moser and Redfern to propose the 5-HT_{1B} receptor subtype being primarily responsible despite the rather contradictory failure of (-)pindolol to antagonize the cue.

Melatonin has not been the subject of a comprehensive drug discrimination study despite evidence in the literature that it

possesses behaviour-modifying properties (see chapter 1, section 1.1.8., this thesis; see also Datta and King, 1980; Redman et al., 1983; Gaffori and Van Ree, 1985). The lack of effect of melatonin in interacting with overt 5-HT mediated behaviour (see chapter 3) prompted the use of the drug discrimination technique which, it was thought, would be more sensitive. It would also serve to investigate the discriminative cue properties of melatonin. The availability of both T-maze and Skinner box paradigms was considered an opportunity to look at whether one technique was more sensitive than the other based on the rate of discriminative cue acquisition. Procedural variations between shock avoidance and hunger relief have been studied before for instance by Krimmer (1974) who noted that drug-appropriate responding using pentobarbital as the training compound continued to apply at lower doses using the shock escape method rather than food pellets. Krimmer also reported that pentobarbital-appropriate responding occurred using sufficiently high doses of alcohol in animals trained by shock escape rather than by hunger relief. Generally however, results obtained in studies of the same drugs with different unconditional stimuli, responses and test situations are remarkably consistent (Kubena and Barry, 1969; Barry, 1974). This implies that drug discrimination is not based on perception of the drug effect on a specific attribute of the response such as its coordination or vigour. Time prevented a more substantial comparison of the cue generated by L-5HTP using the T-maze (shock avoidance) paradigm as opposed to the Skinner box (hunger relief) technique. The use of the Skinner box it was thought would yield additional data with respect to melatonin concerning its possible effects on an FR schedule. Although not covered in this thesis there is also a need for a more substantial investigation

into the effects of melatonin on performance in an operant conditioning context. Melatonin is thought to act as a hypnotic which would imply a non-specific discriminative stimulus in the sedative/tranquillizer class of drugs.

The opportunity was also taken to investigate whether or not the discriminative properties of L-5HTP displayed a circadian rhythm. In addition to the general chronobiological interest that a positive result would initiate, it was felt that the absence of a rhythm would provide circumstantial evidence in support of two aspects of 5-HT research. These are: firstly, 5-HT_1 receptor activity does not express a circadian rhythm; and secondly, the 5-HT_2 receptor is not involved in the discriminative properties of L-5HTP. If a positive result were obtained then further investigation would be necessary in order to assess whether either of these two hypotheses are correct or or if another factor is involved. The experiment was performed using rats whose discriminative ability with regard to L-5HTP versus saline had been well-established. Moser (1986) had previously obtained a negative result when he investigated the potential for the L-5HTP cue to display a circadian rhythm by training the animals at six different time points. The assumption was that the rate of cue acquisition would vary according to the time of training if a rhythm was present. This method did not test a possible diurnal variation in the cue with trained rats. It is possible that a cue assumes different perceptual characteristics between novice and experienced animals.

4.2. Materials and Methods

4.2.1. Drugs

All drugs were given in normal saline. At the 1mg/kg dose melatonin (Sigma) was dissolved by sonication. Doses 10mg/kg and 50mg/kg were suspended in 0.1% w/v tragacanth in normal saline by sonication. (L-5HTP (Sigma), 5MeODMT (Sigma) and 8-OHDPAT (Research Biochemicals Incorporated) were dissolved by gentle heating. Carbidopa (M.S.D.) was suspended in normal saline by sonication. All drugs and saline controls were injected i.p. using a dose volume of 5ml/kg, except 8-OHDPAT which was injected s.c. using a dose volume of 2.5ml/kg.

4.2.2. Apparatus

Standard operant conditioning chambers, containing two levers, were used (Campden Instruments) as shown by Fig. 4.1. A reinforcement delivery chamber was located between the levers. The liquid food reinforcement consisted of 0.02ml of sweetened condensed milk:water 1:1 dilution delivered by a motorized dipper from a food reservoir located below. Presentation of the food and recording of behavioural responses was achieved using a BBC Master computer in conjunction with a SPIDER cartridge (Paul Fray Ltd.) and software designed and written by the author (for details see Appendix). The T-maze (Fig. 4.2) consisted of 7-plywood walls 30cm high and an electrified (0.5mA) grid floor. The floor of the hidden safe areas located at either ends of the arms of the T were constructed of 7-plywood and admission to them could be prohibited using a wooden gate. Deliverance of the shock was achieved using a constant current shock generator (Campden Instruments, Model 521C) in conjunction with a scrambler (Campden Instruments, Model 521S).

Fig. 4.1. Diagram showing the main features of the operant conditioning chamber (Skinner Box) used in the drug discrimination and light-dark studies.

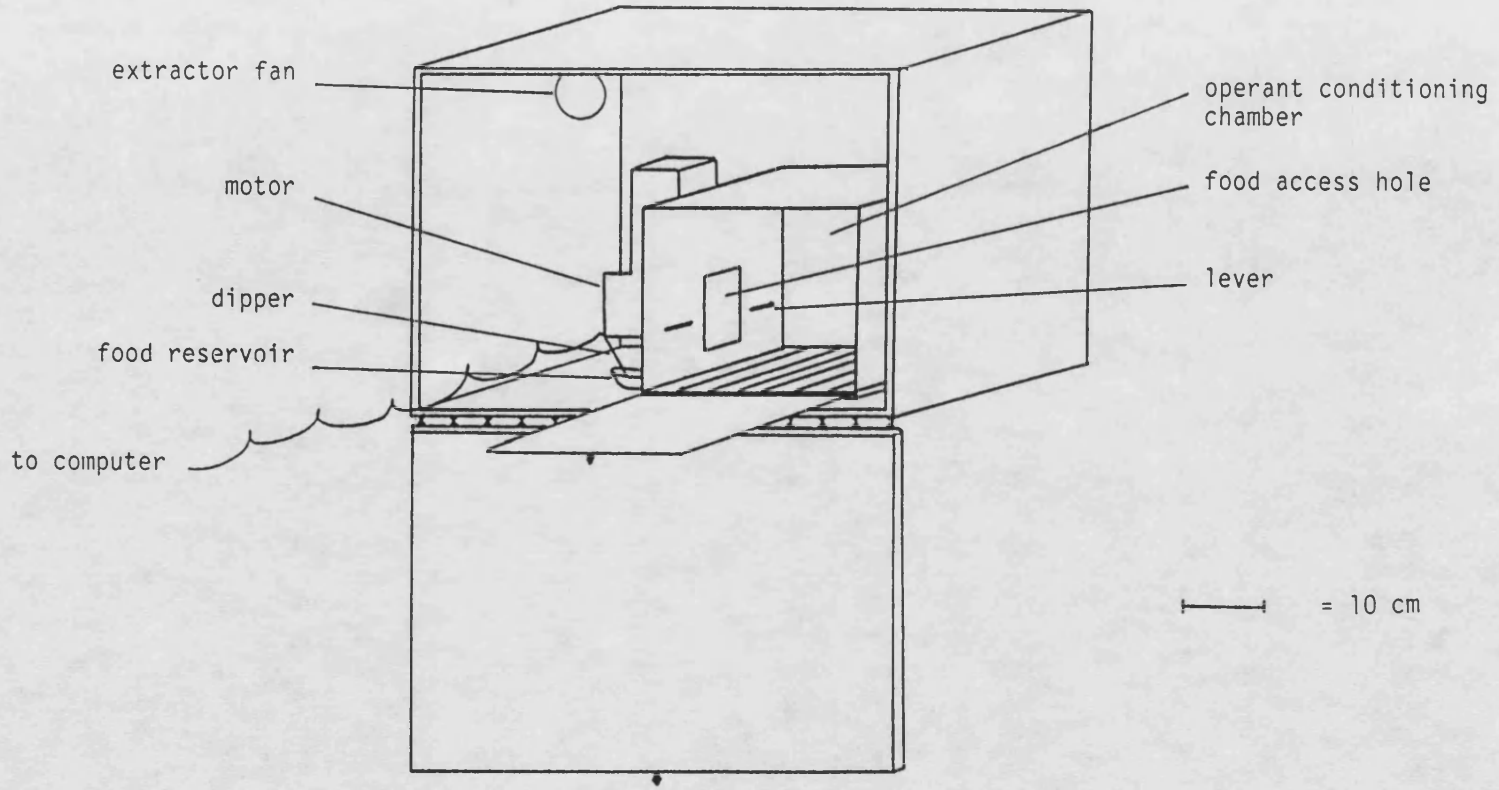


Fig. 4.2. Diagram of the T-maze used in the drug discrimination studies.

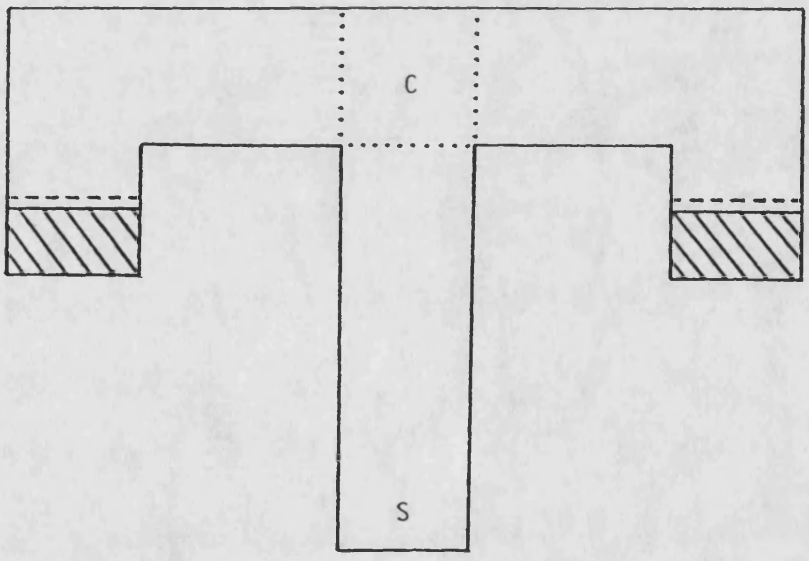
= safe area

S = start point

C = choice point

---- = position of barriers

⟷ = 10 cm



4.2.3. Animals

Male Wistar rats (Animal House, University of Bath strain) weighing between 150 and 200g at the start of the experiment were housed in groups of five in standard cages (520 x 350 x 170mm), on a LD cycle of 12:12 (lights on 05:00 h). Water was freely available both to rats to be trained via the T-maze and to those trained via the Skinner box paradigm. Food (Labsure CRM diet) was freely available to those trained using the T-maze apparatus.

4.2.4. Training procedure for Skinner box paradigm

Rats were gradually acclimatized to a restricted diet schedule comprising of a 2 h food presentation period commencing just after the final training session (17:00 h). Training sessions were conducted at the EOL stage between the 13:00 h and 17:00 h. The weight of the animals was monitored every week and their general condition and health observed daily. One rat had to be withdrawn due to middle-ear infection. Once lever-pressing ability had been established using continuous reinforcement (i.e. each lever press was reinforced), training sessions lasting 15 min were introduced and the contingency was changed such that only the correct lever was rewarded if pressed a pre-programmed number of times. Once familiar with this task the FR contingency was gradually increased until response on a FR10 schedule was satisfactory. At this point discrimination training began in earnest, with each session limited to 15 min The procedure is similar to that required for discrimination training using the T-maze paradigm. Rats are required to press one lever for a food-reward under the influence of saline and the other when given drug. Half of a group are trained to press, for example, the left lever when given saline and half the right

lever to control for positional preferences. To control for potential odour cues influencing lever choice (Extance and Goudie, 1981) after each training session the levers were wiped with alcohol. One group of rats were trained to discriminate 5-HTP (35mg/kg) from saline and another group melatonin (1, 10 and 50mg/kg) from saline. The group receiving melatonin were given 20 training sessions at each dose, the 10 and 50mg/kg sessions required the use of saline/tragacanth mixture in place of saline on the appropriate non-drug-training days. Once a day training sessions were conducted 5-7 days a week, drug and saline days being randomly arranged such that for no more than two successive sessions did animals receive the same treatment. Animals were injected with the appropriate drug 30 min before being put into the chamber. The group receiving L-5HTP as the training drug were pretreated with carbidopa (25mg/kg) 60 min prior to the start of a training session on both saline and drug days. Animals were deemed to have reached criterion if they made 8 correct choices in 10 consecutive sessions. Once discrimination had been established a dose-response curve was constructed.

4.2.5. Training procedure for T-maze paradigm

Animals were initially trained to avoid the electrified grid floor of the T-maze by turning left or right at the "choice point" to reach a hidden safe area. Once familiar with the procedure, discrimination training began. The concept involved is the same as that used for the Skinnerian discrimination procedure outlined above. Rats are required to turn in one direction if given saline and in the other if given drug. Positional preferences were controlled for as described above, and odour cues by wiping the grid

floor with alcohol after each rat's training session. Training sessions consisted of 8 trials 1 min apart. Rats trained to discriminate L-5HTP from saline were given carbidopa (25mg/kg) 60 min prior to the training session and either L-5HTP (50mg/kg) or saline 30 min before the start of the session (Moser and Redfern, 1985b). The threshold for criterion was set at 8 correct choices in 10 consecutive sessions. Once discrimination had been established a dose response curve was constructed. Animals in the melatonin training sessions were given either saline or melatonin, 1mg/kg, 10mg/kg or 50mg/kg, 30 min before the start of the session. The threshold for criterion was again designated as 8 correct responses in 10 consecutive sessions.

4.2.6. Testing of stimulus generalization and antagonism

Experiments were carried out on Tuesdays and Fridays once animals had achieved the discrimination criterion; training continued on the other days to maintain discriminative stimulus control. If the performance of a rat fell below the criterion level then it was withdrawn from experiments until the appropriate level had been restored. For test sessions involving the Skinner box paradigm animals were put into the chambers for 5 min only during which time either lever, if pressed ten times, would provide reinforcement. The first lever to be pressed ten times was designated the chosen lever and became the reinforcement lever. Responses on the other lever were recorded but had no programmed consequences. Only a dose-respnse curve to L-5HTP was constructed using this apparatus. To prevent animals from being able to differentiate between training and experimental sessions due to their unequal duration, they were put into a "neutral" plastic cage

(420 x 260 x 150mm) immediately after the experiment for the 10 min remaining of an equivalent training session before being returned to the home cage.

The T-maze paradigm was used to establish the dose-response curve and time-response curve to L-5HTP and also characterization of the L-5HTP cue itself. Experimental sessions consisted of only one trial during which either safe area could be reached. The chosen direction was designated that which the animal entered up to the base of its tail. Generalization studies were carried in the absence of carbidopa using 5MeODMT (0.5, 1, 2, 4 mg/kg), using a 10 min pretreatment time, 8-OHDPAT (0.5, 1, 2, 4 mg/kg) using a 30 min pretreatment time and melatonin (1, 10, 50 mg/kg) also with a 30 min pretreatment time. An antagonism study was conducted using melatonin (1, 10, 50 mg/kg) given immediately after the L-5HTP injection, i.e. 30 min before the start of the experimental session.

4.2.7. Investigation into the discriminative ability of L-5HTP over 24 h.

Only the T-maze was used in this experiment. A second dose response curve was constructed in animals whose ability at distinguishing 50mg/kg L-5HTP from saline was well-established. Using the approximate ED_{50} derived from this curve and two additional doses, one above and one below the ED_{50}, the discriminative ability of the animals was tested at four time points of the LD cycle in sessions starting 4.5, 10.5, 16.5 and 22.5 h after lights on.

4.3. Data Analysis

Discrimination during training was expressed as a percentage of correct choices in 5 consecutive sessions with respect to both the T-maze and Skinner box paradigms. During training the "Vicarious Trial and Error" (VTE) characteristic was measured. This is said to occur when, on the first trial of a training session the animal halts at the choice point and looks down both arms of the "T" before making a choice as if trying to decide or remember which route might lead to the safe area. It is used to define the confidence displayed by an animal in its reinforcement choice with respect to the T-maze paradigm. The FRF value (ratio of correct lever presses against incorrect lever presses until the FR number is reached on the reward lever expressed as a percentage of the total) was used as a measure of this "confidence" parameter for the Skinner box. The Skinner box was able to provide additional data in the form of response latency and response rate. The latter was defined as the rate of lever presses on the correct lever per min after the first reinforcement; response latency is the time taken for an animal to press the correct lever the appropriate number of times as defined by the FR ratio in this context. Since two Skinner boxes were operative both possessing non-retractable levers response latency was measured on alternate animals for each daily training session - rats belonging to the designated group were put into their experimental chamber first and the timer started. The rat from the second group was then put into its chamber and the response latency factor ignored. Graphically this is expressed as the mean response latency derived from two successive sessions of the same treatment. During test sessions only the response rate was measurable.

4.4. Results

4.4.1. Acquisition of the L-5HTP/saline discriminative stimulus

Animals trained on both paradigms achieved satisfactory discriminative ability though it took those animals using the T-maze paradigm considerably longer than their Skinner box counterparts. T-maze rats took upwards of 50 (mean = 53 +/- 3.9 s.e.m.) sessions to reach criterion while the Skinner box rats took 20 sessions (mean = 20.7 +/- 2.3 s.e.m.). The acquisition curves to the L-5HTP cue are shown in Fig. 4.3a (T-maze) and 4.3b (Skinner box). The dose response curves of L-5HTP for each paradigm are depicted in Figs. 4.4a and 4.4b.

4.4.2. Acquisition of the melatonin/saline discriminative stimulus

Only one rat in the Skinner box group managed to achieve criterion but this proved unstable. The overall failure of the group to achieve a satisfactory and reliable response at 1mg/kg led to the susbsequent increments in melatonin dosage to 10mg/kg and 50mg/kg, after giving 20 sessions to the animals at each dosage. None of the rats in the T-maze group achieved criterion, of which there were two groups; those treated with melatonin 1mg/kg for 50 sessions and those treated with 10mg/kg for 40 sessions initially, the dose then being increased to 50mg/kg for a following 70 sessions. The training plots for melatonin are shown in Fig. 4.5a (T-maze) and 4.5b (Skinner box) which, for comparative purposes, also shows the training plots for L-5HTP. Acquisition curves for melatonin are shown in Figs. 4.6a (melatonin 1mg/kg, T-maze), 4.6b (melatonin 10 and 50mg/kg; T-maze) and 4.6c (melatonin 1, 10 and 50mg/kg; Skinner box).

4.4.3. Compounds generalizing to the L-5HTP discriminative stimulus

Only the T-maze paradigm was used in this study. At the highest doses used 8-OHDPAT and 5MeODMT produced discriminative cues indiscernible from L-5HTP (see Fig. 4.7). All doses of melatonin, however, induced saline-appropriate responding (see Fig. 4.8).

4.4.4. Antagonism study of the L-5HTP cue using melatonin

Only the T-maze paradigm was used in this study. Melatonin failed to antagonize the L-5HTP discriminative cue at all the doses employed (see Fig. 4.8).

4.4.5. Effect of L-5HTP and melatonin on response latency, rate of response and the FRF characteristic on the correct Lever

For obvious reasons only the Skinner box paradigm yielded data to this behavioural characteristic. Once training had become established, L-5HTP training days displayed no difference to saline training days. However, the first two L-5HTP training sessions heralded a major inhibition in responding (see Fig. 4.9) with only four rats attaining the FR value after a long period of nil responding. This prompted a reduction in the initial L-5HTP dose used from 50mg/kg to 35mg/kg. Melatonin had no effect on the response latency parameter (see Fig. 4.10). L-5HTP also suppressed the overall response rate throughout the course of the discriminative training. Once again melatonin had no effect (see Fig. 4.11). The rapid acquisition of the L-5HTP cue is demonstrated in Fig. 4.12 which plots the FRF value for saline and drug days.

Fig. 4.13 depicts the dose response curves for the FRF value for the drug-appropriate lever and the response rate obtained

during the determination of the L-5HTP discriminative stimulus dose response curve using the Skinner box paradigm.

4.4.6. Effect of time-of-day on the cue-generating properties of L-5HTP

No discernible diurnal variation in the response to L-5HTP was obtained at any of the dose levels used (see Fig. 4.14).

Fig. 4.3a. Acquisition of discrimination between saline and L-5HTP (50mg/kg) using the T-maze paradigm. (n=11). Solid line = drug training days, dashed line = saline training days, ■ = VTE characteristic.

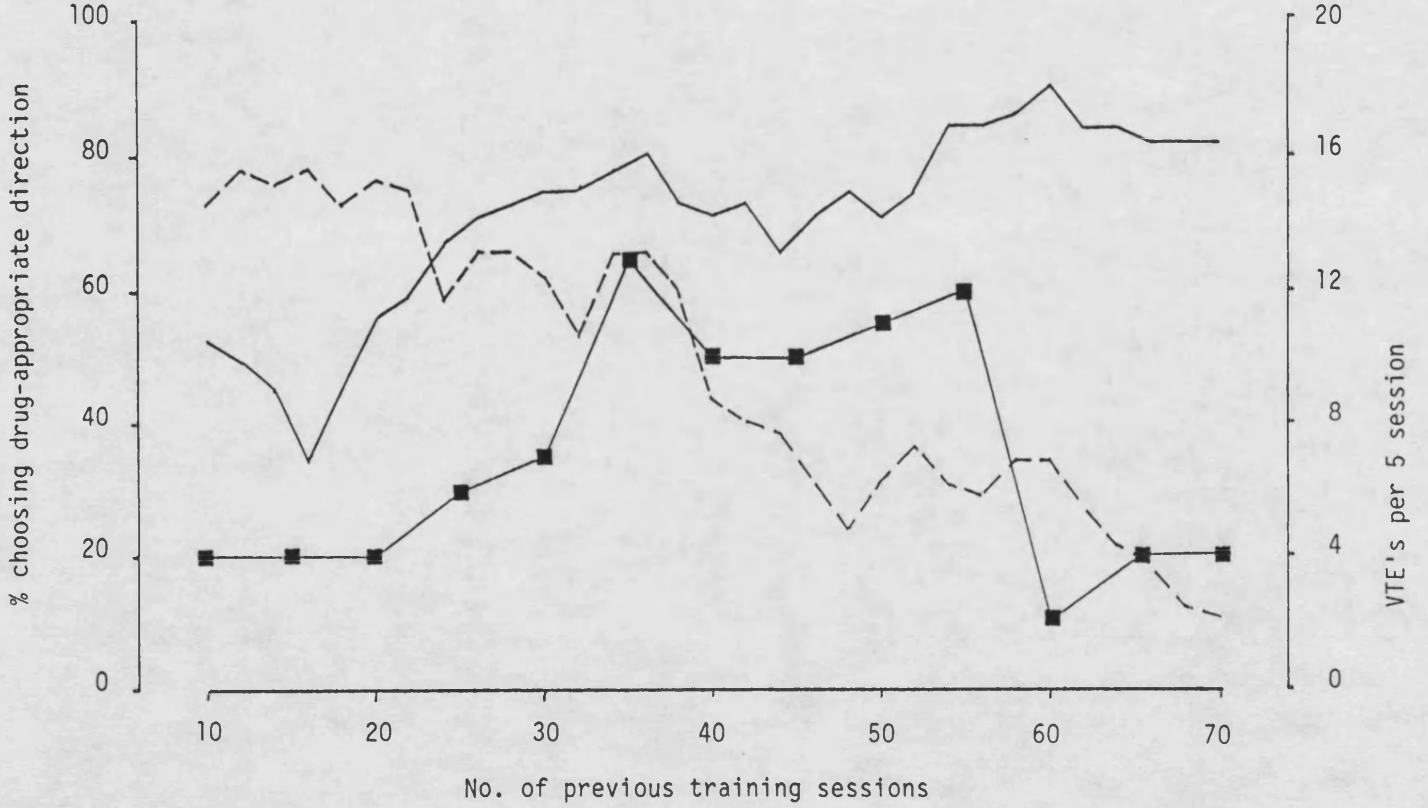


Fig. 4.3b. Acquisition of discrimination between saline and L-5HTP (35mg/kg) using the Skinner box paradigm. (n=7).

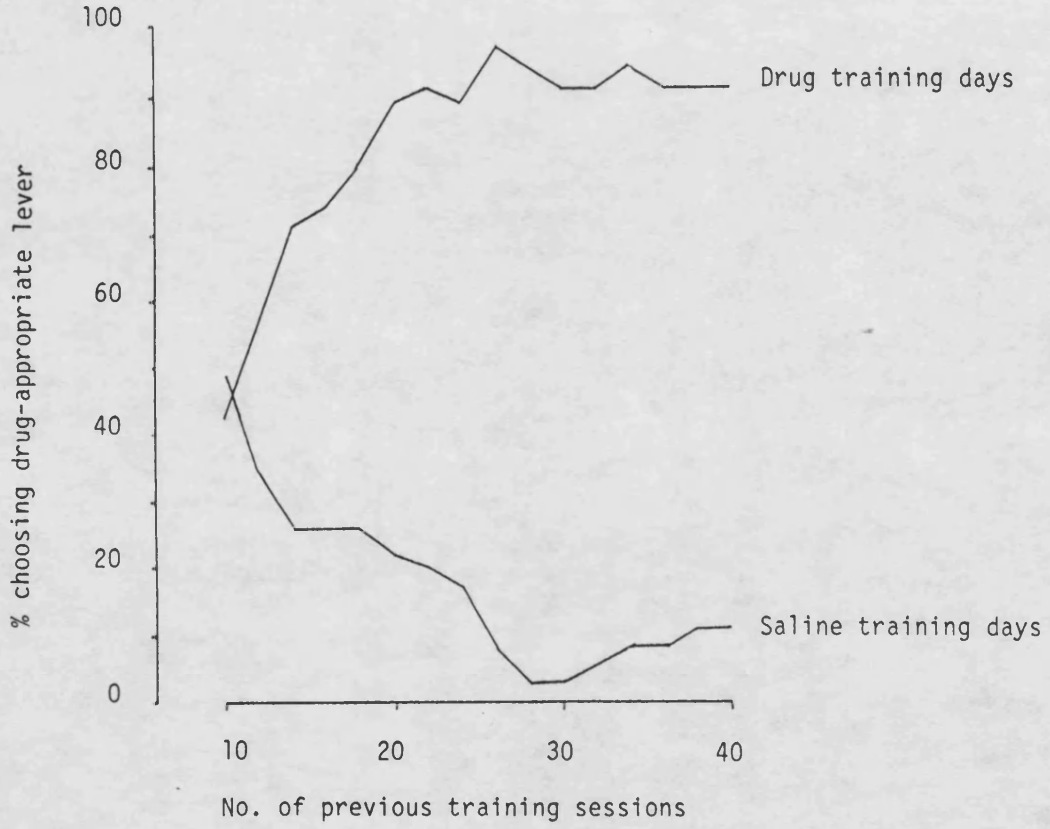


Fig. 4.4. Dose response curves of L-5HTP for (a) Skinner box paradigm (training dose = 35mg/kg, n=7) and (b) T-maze paradigm (training dose = 50mg/kg, n=11).

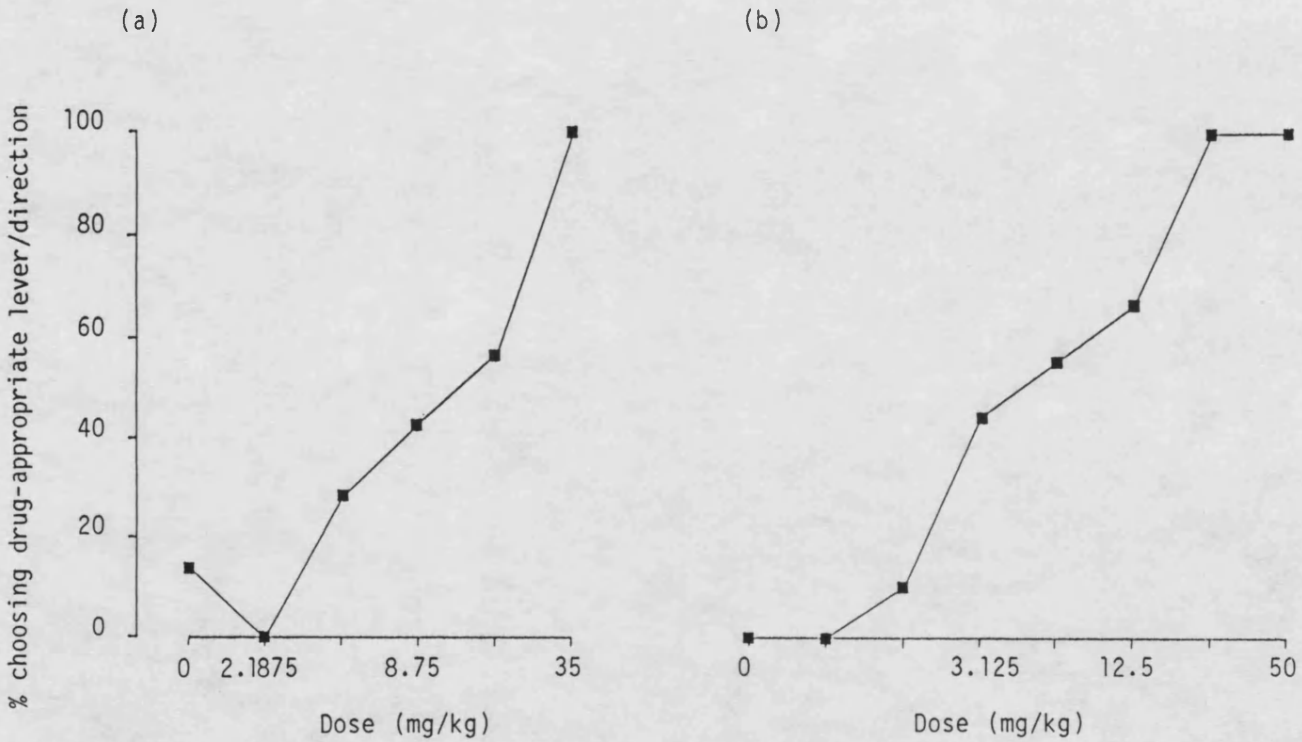


Fig.4.5a. Training curve to melatonin and L-5HTP using the T-maze paradigm.

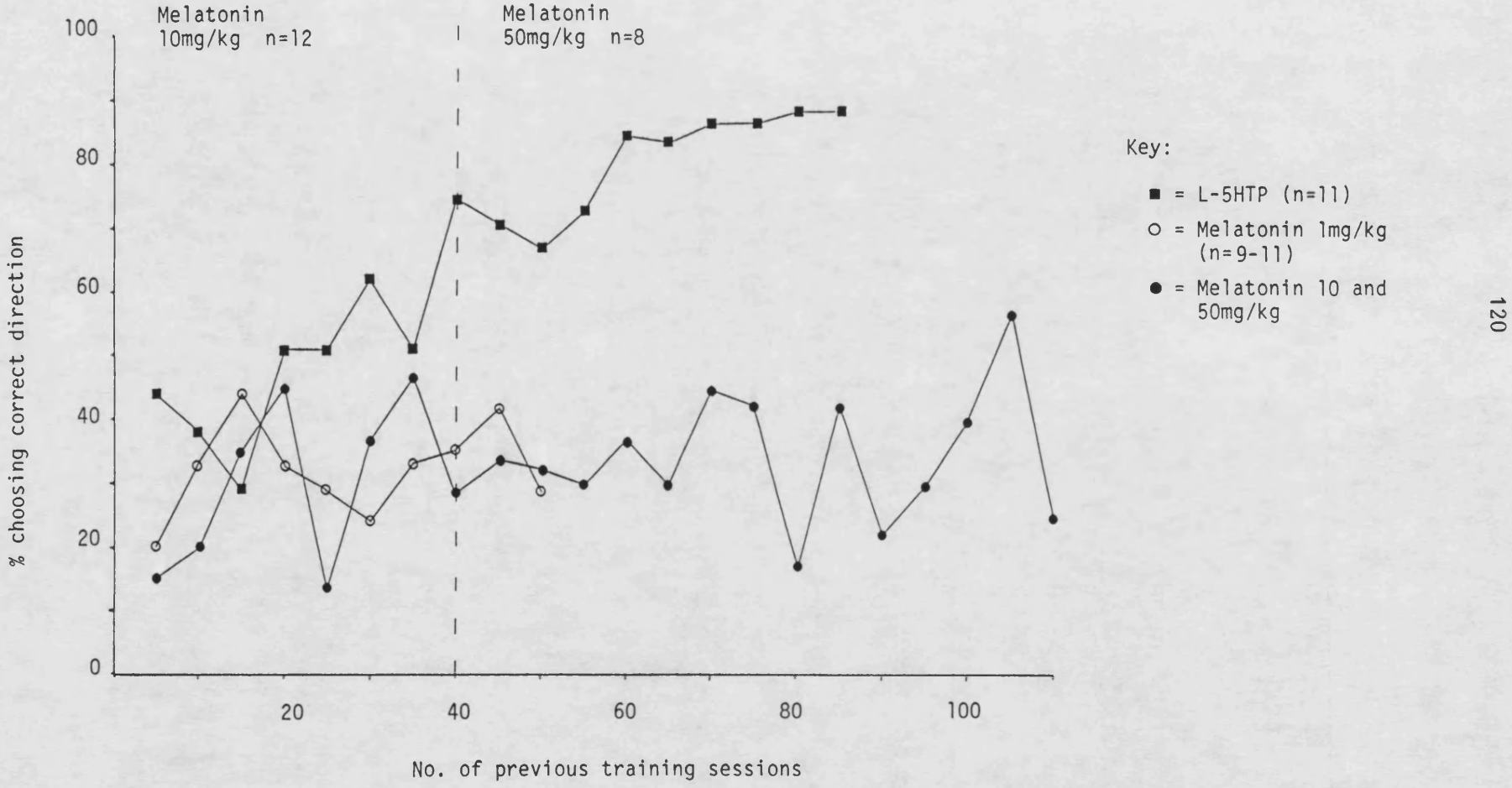


Fig. 4.5b. Training curve to melatonin and L-5HTP using the Skinner box paradigm.

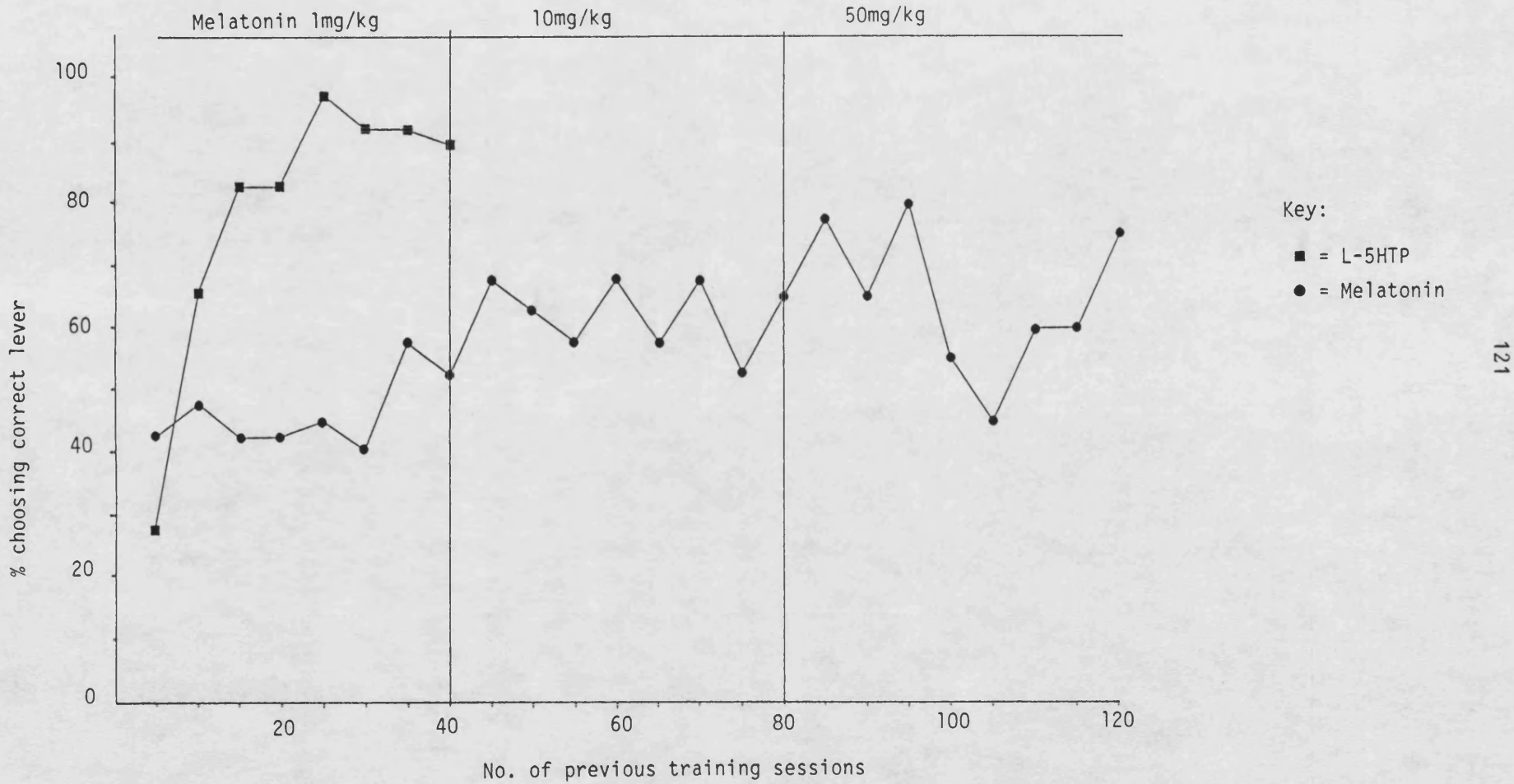


Fig. 4.6a. Acquisition of discrimination between saline and melatonin (1mg/kg) using the T-maze paradigm. (n=9-11). Solid line = drug training days, dashed line = saline training days, ■ = VTE characteristic.

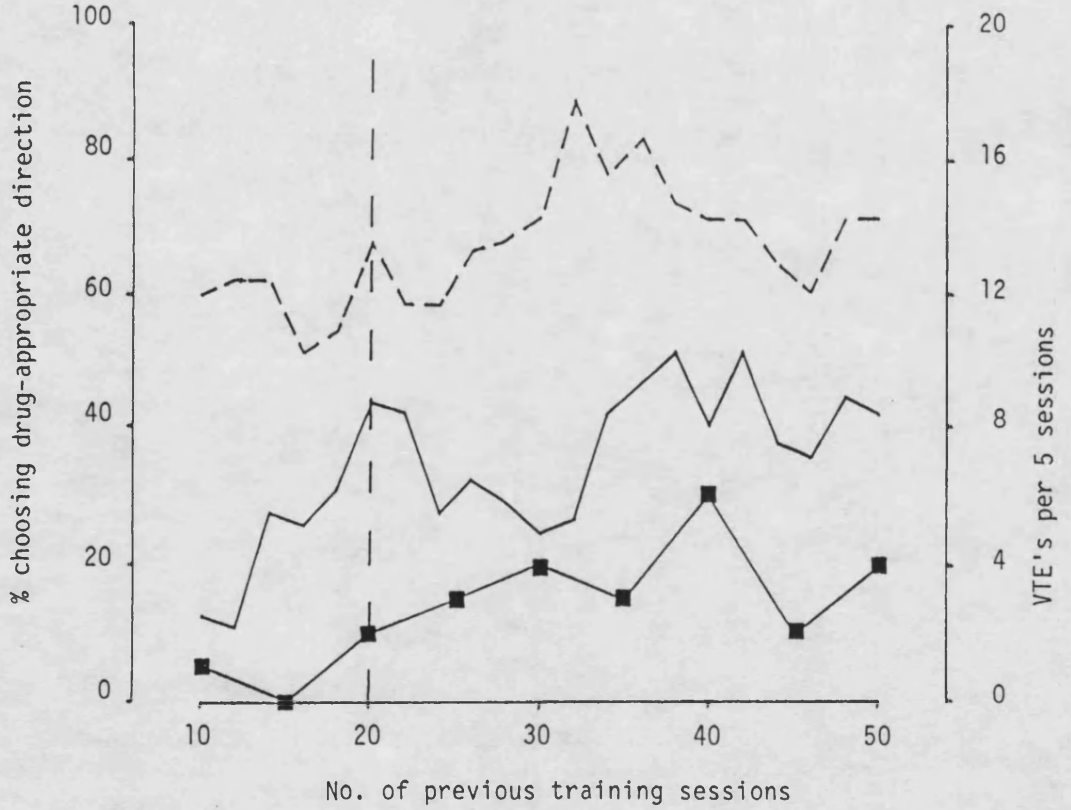


Fig. 4.6b. Acquisition of discrimination between melatonin (10 and 50mg/kg) using the T-maze paradigm. (n=9-11). Solid line = drug training days, dashed line = saline training days, ■ = VTE characteristic.

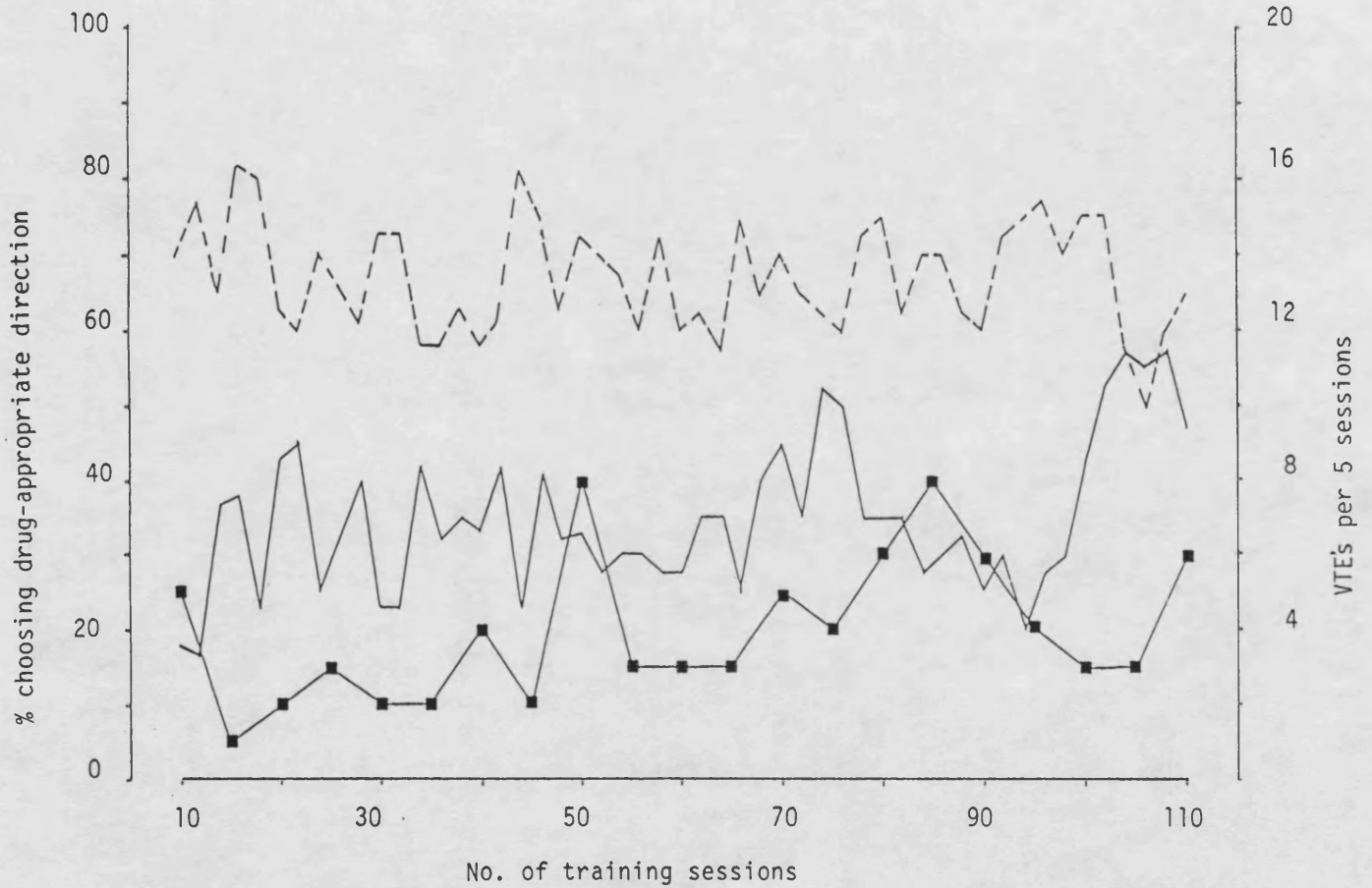


Fig. 4.6c. Acquisition of discrimination between melatonin (1, 10 and 50mg/kg) using the Skinner box paradigm. Solid line = drug training days, dashed line = saline training days. (n=8).

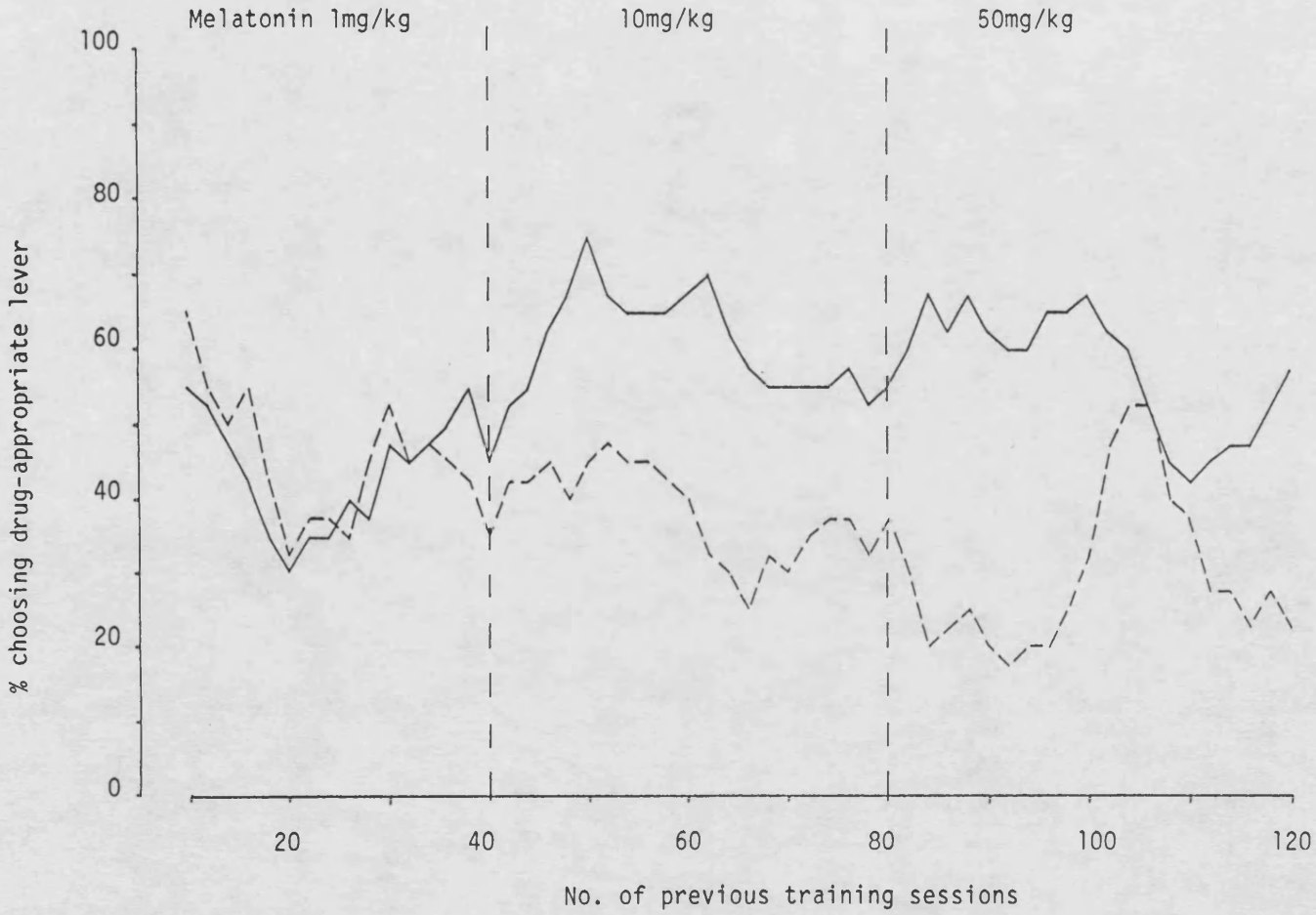


Fig. 4.7. Discriminative cue properties of L-5HTP - generalization to 8-OHDPAT and 5MeODMT.

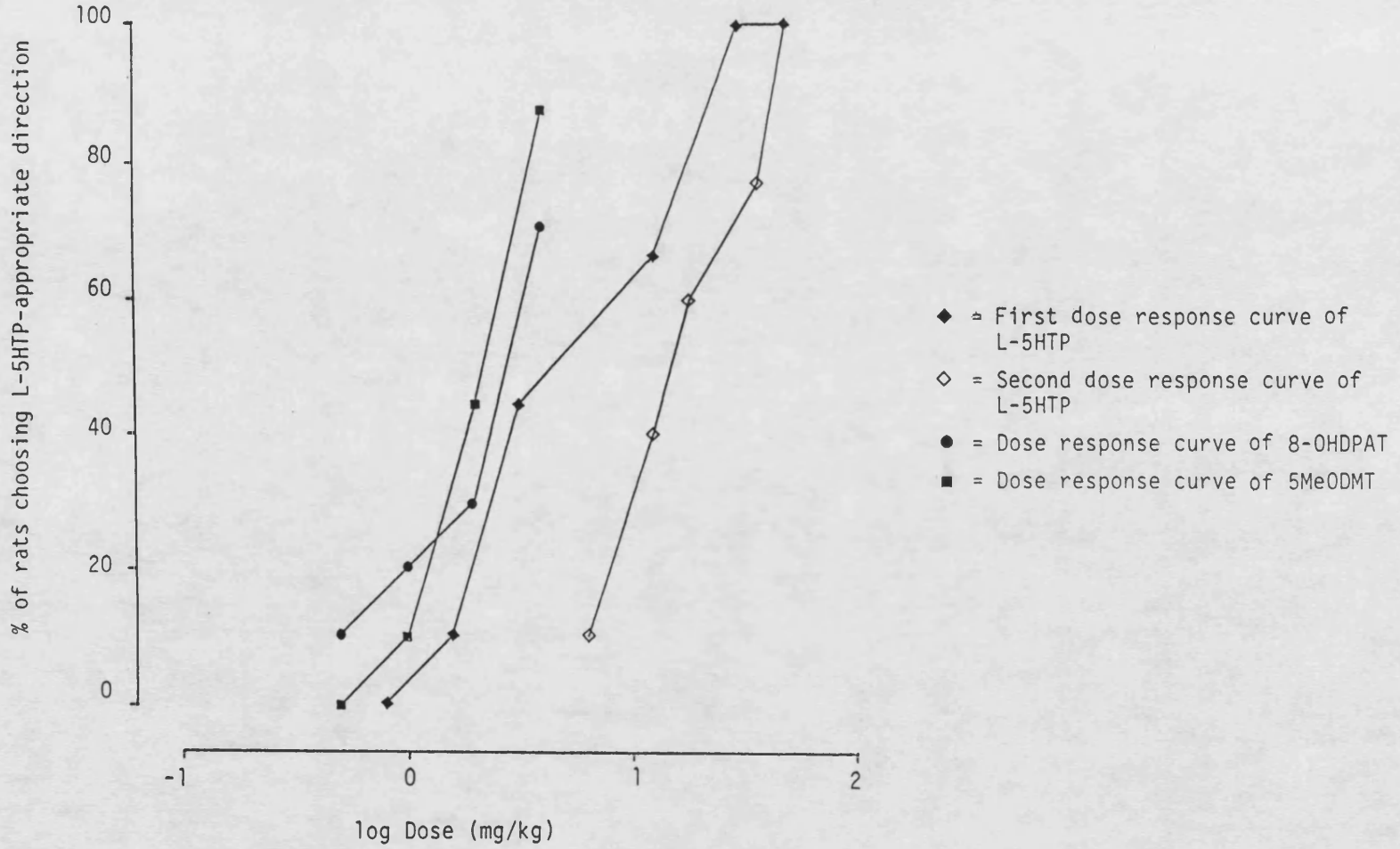


Fig. 4.8. Melatonin and the discriminative cue properties of L-5HTP. (n=10).
■ = substitution of L-5HTP by melatonin
▼ = antagonism of L-5HTP (50mg/kg) by melatonin

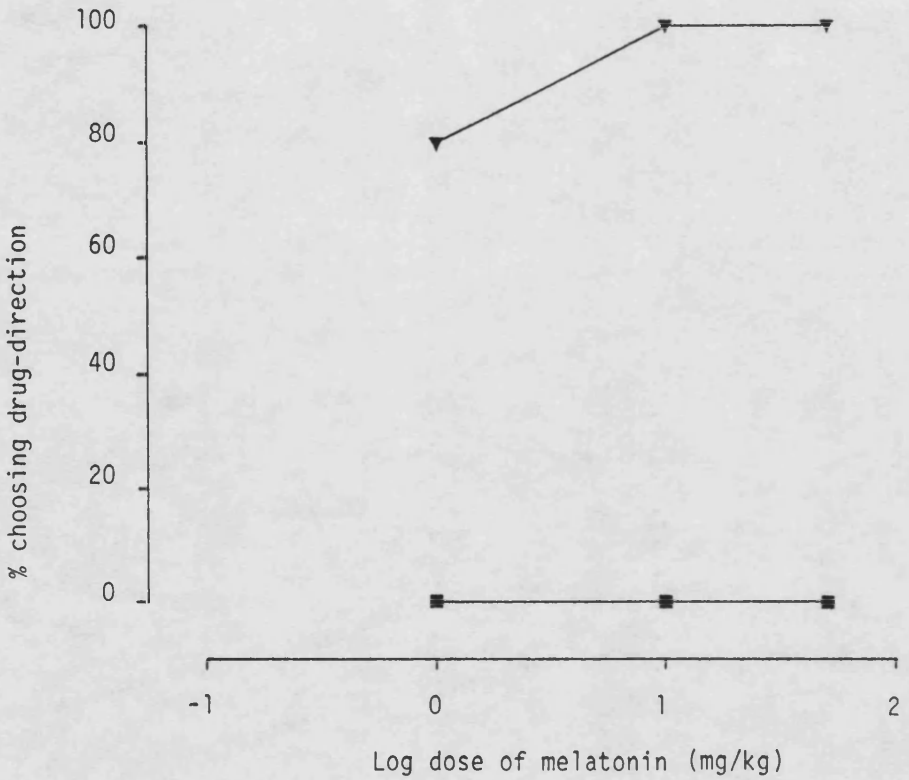


Fig. 4.9. Comparison of response latency times between rats undergoing training to discriminate L-5HTP (35mg/kg) from saline and rats undergoing training to discriminate melatonin (1mg/kg) from saline. (Values represent means of 4-8 rats).

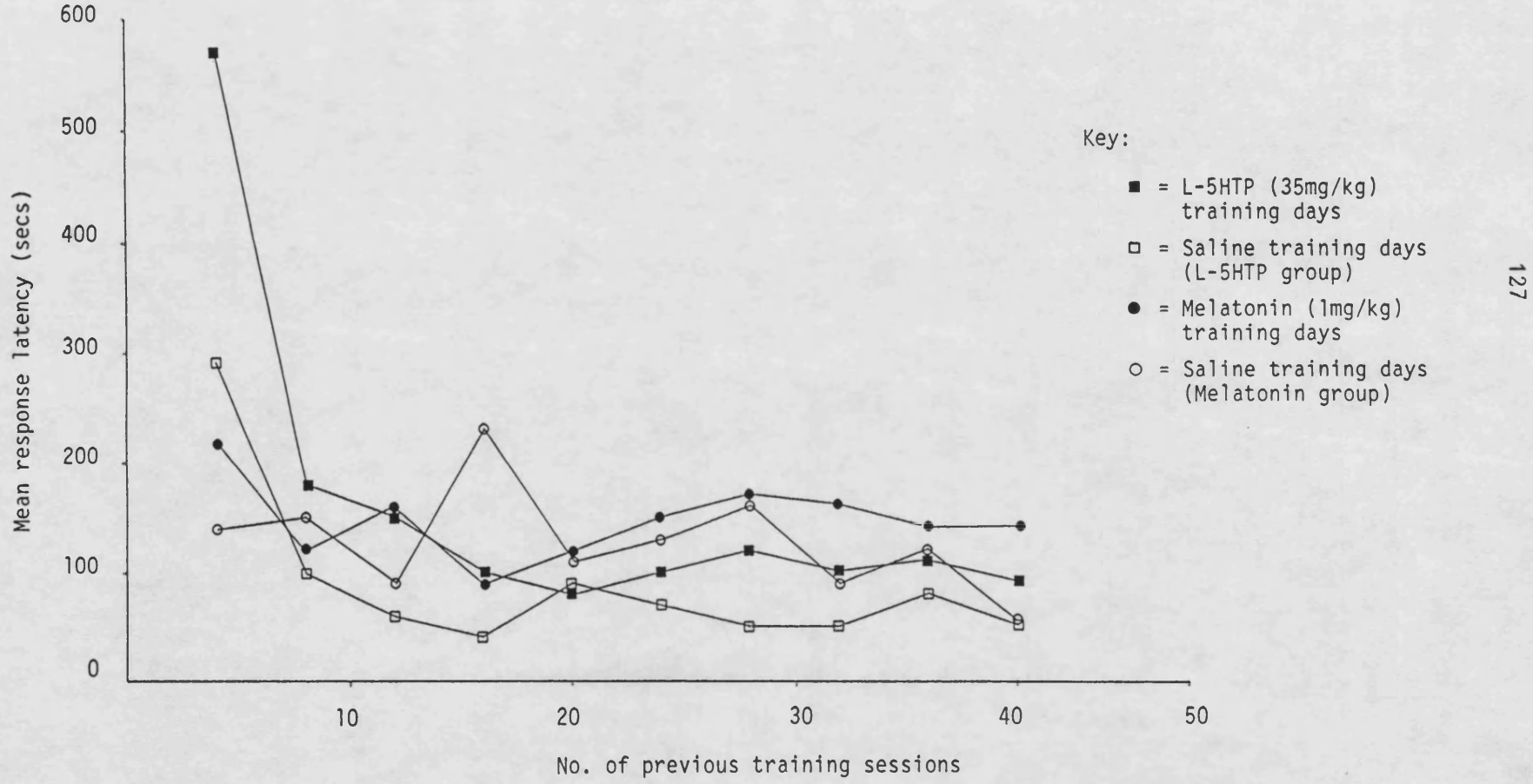


Fig. 4.10. Comparison of response latency times between rats undergoing training to discriminate melatonin (1, 10 and 50mg/kg) from saline. (Values represent means of 6-8 rats).

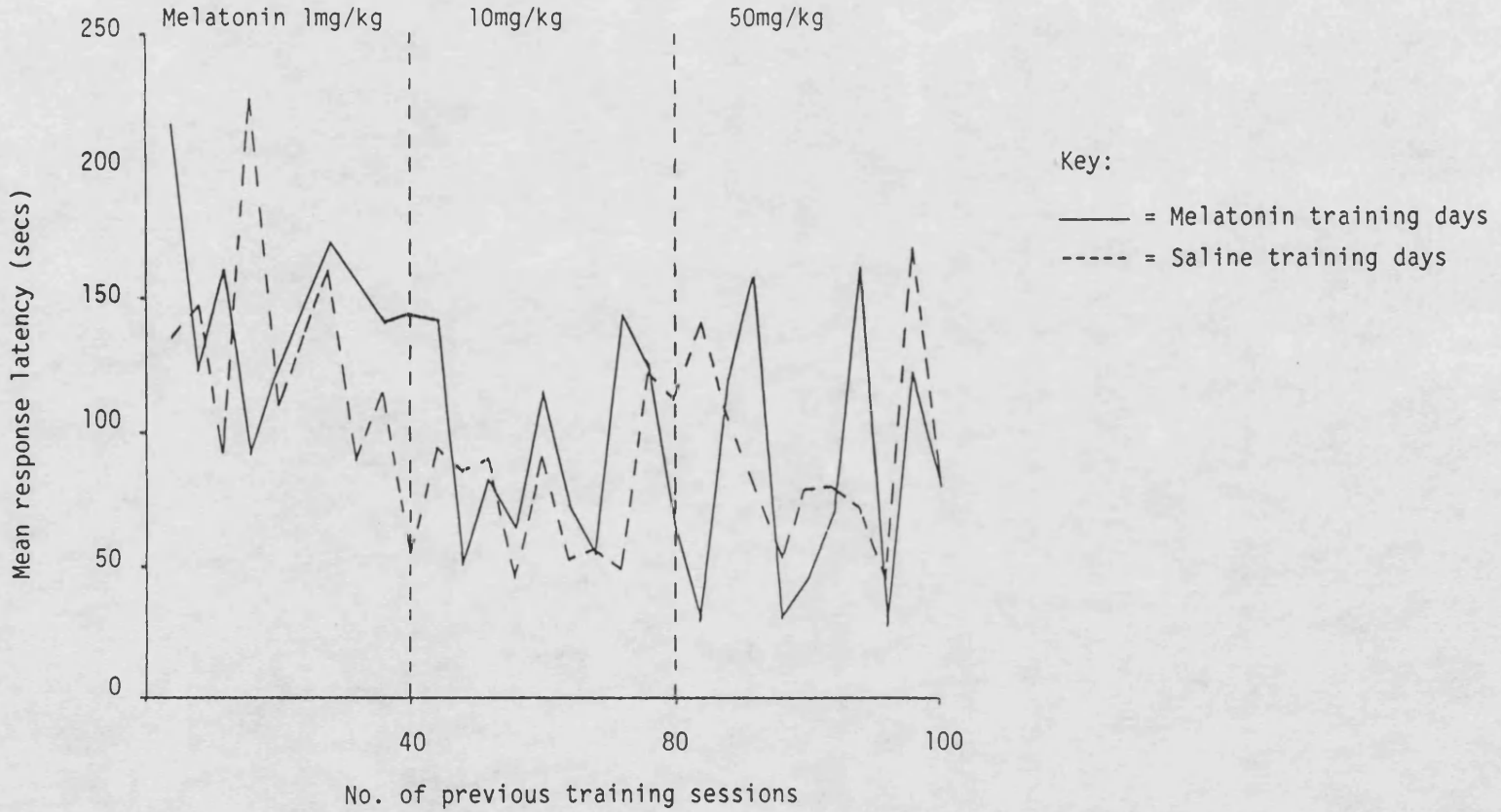


Fig. 4.11. Comparison of mean response rates (lever presses/min) between rats undergoing training to discriminate L-5HTP (35mg/kg) from saline and rats undergoing training to discriminate melatonin (1, 10 and 50mg/kg) from saline. (Values represent means of 6-8 rats).

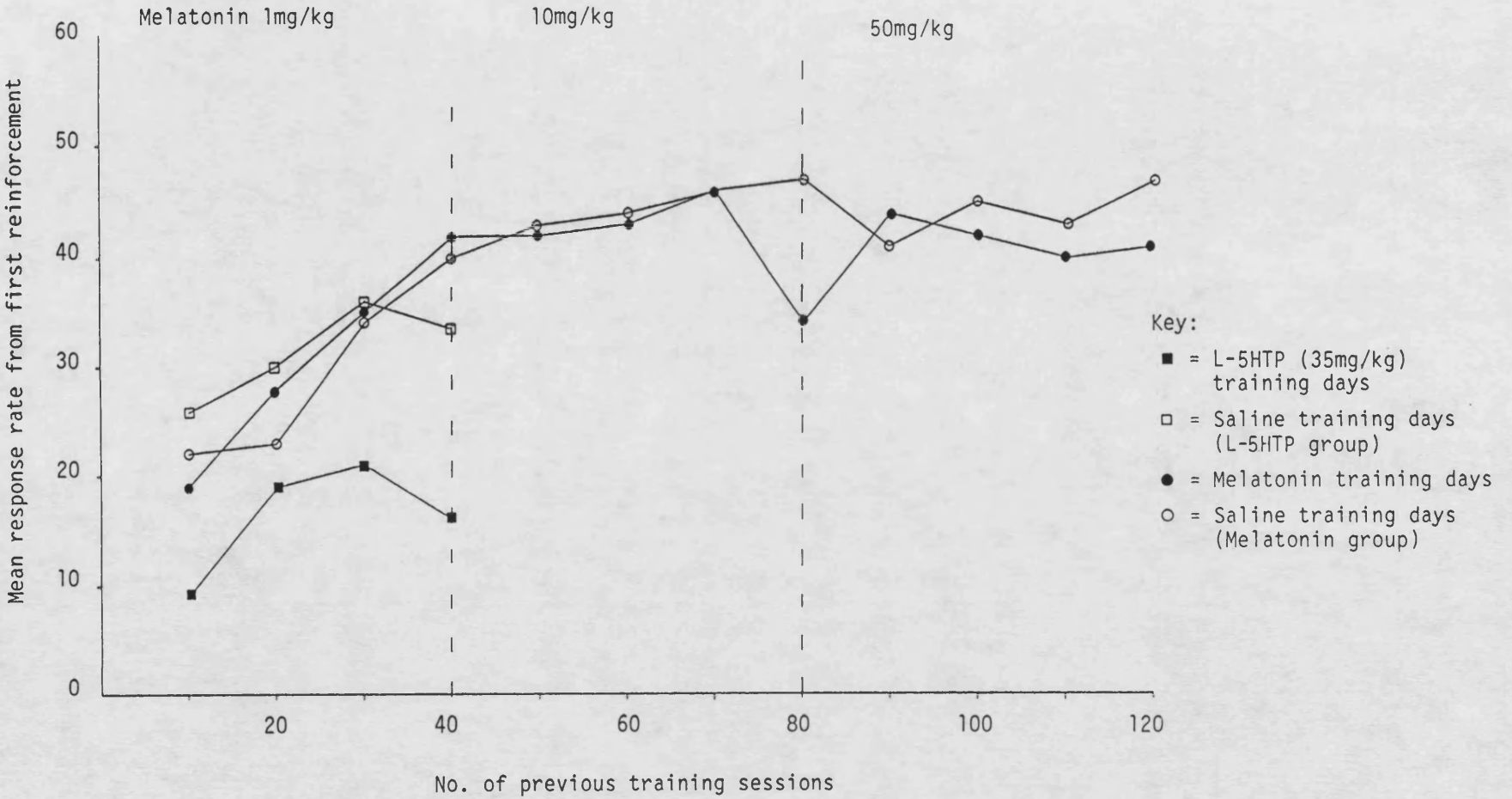
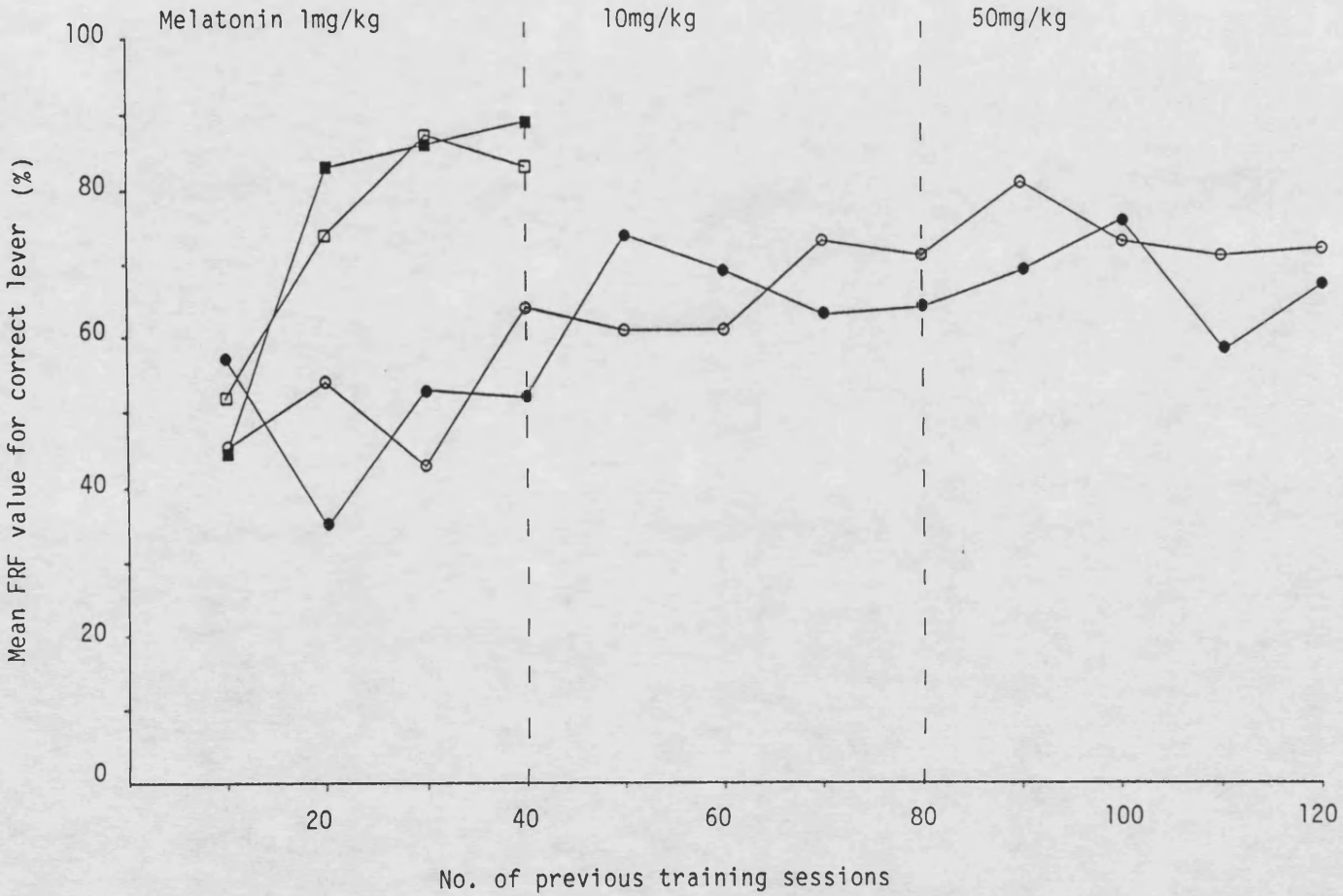


Fig. 4.12. Comparison of FRF values for rats undergoing training to discriminate L-5HTP (35mg/kg) from saline and rats undergoing training to discriminate melatonin (1, 10 and 50mg/kg) from saline. (n=6-8).



Key:
As for Fig. 4.11.

Fig. 4.13. Dose response curve for rate of response and drug-appropriate FRF value in rats trained to discriminate L-5HTP (Training dose = 35mg/kg) from saline. Open circles = rate of response (lever press/min), closed circles = FRF value. (n=7).

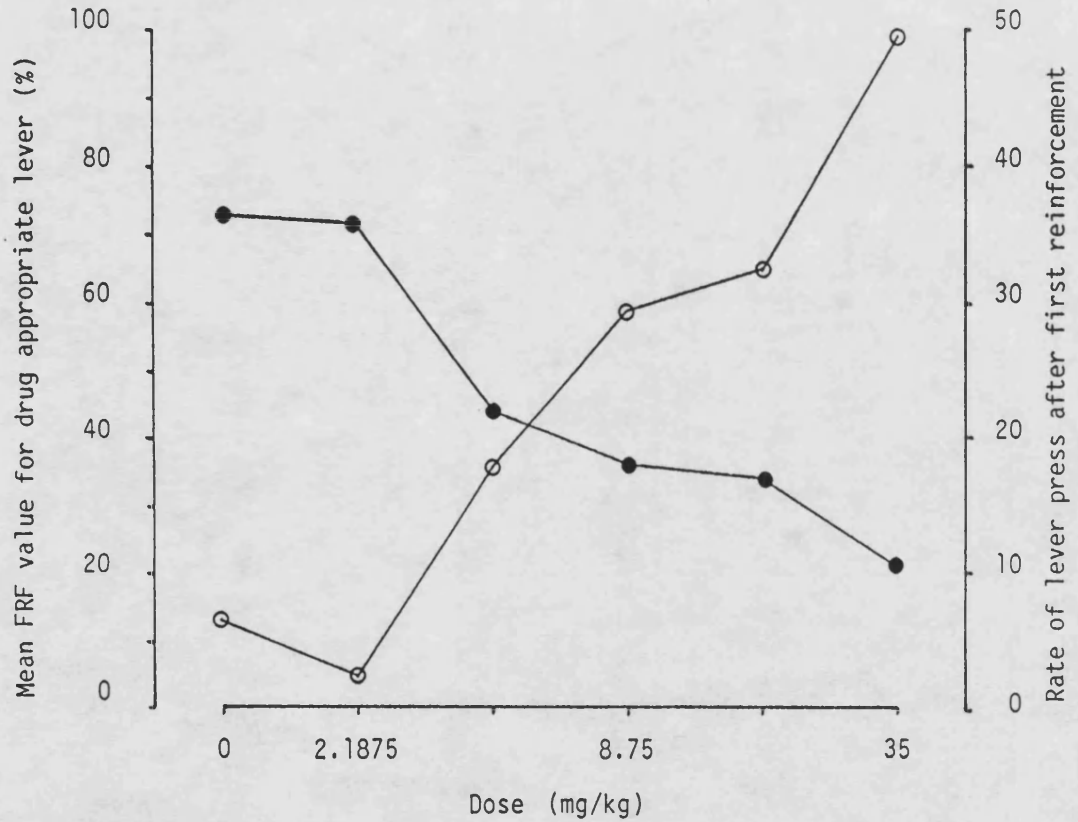
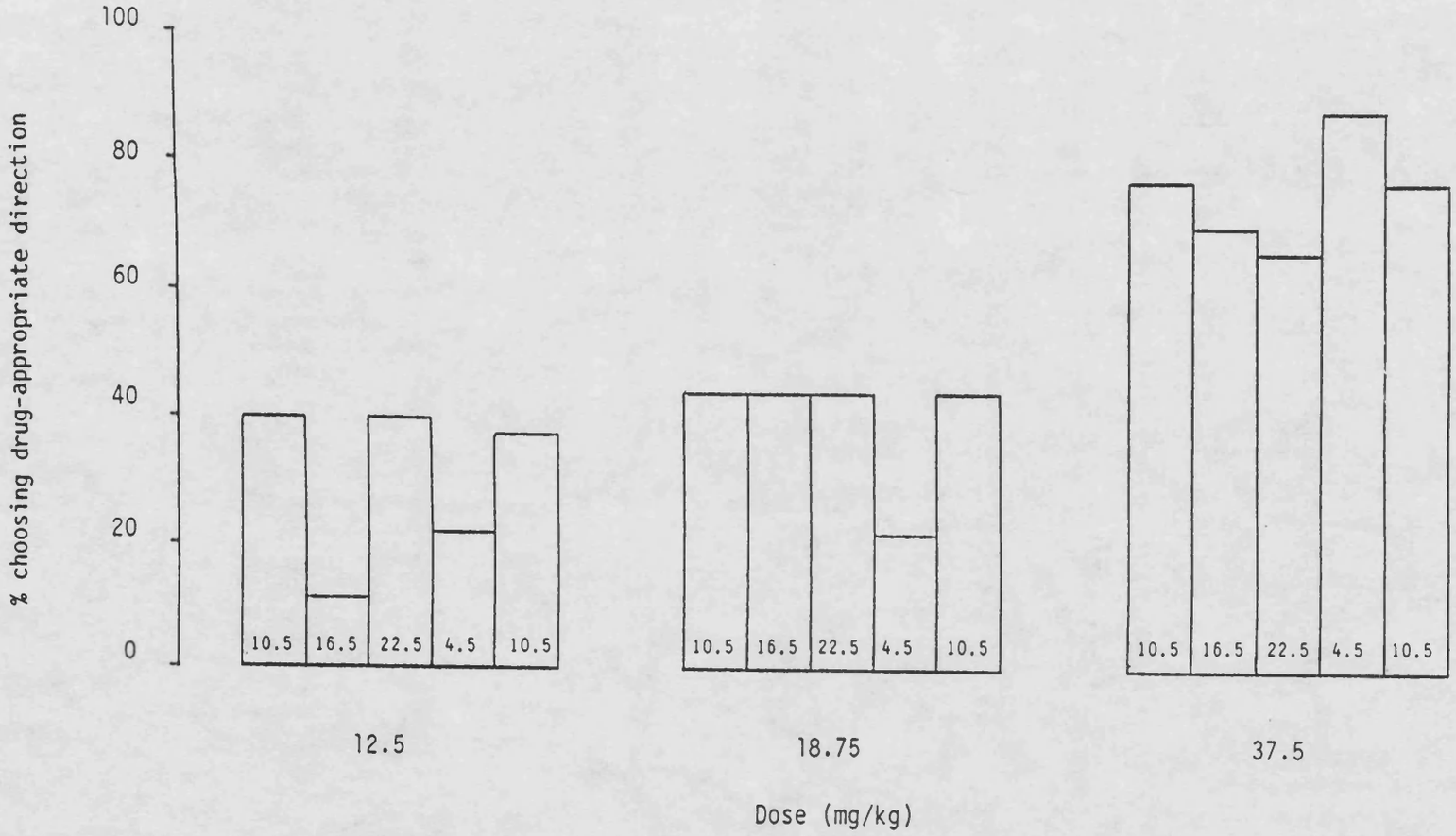


Fig. 4.14. Effect of time-of-day on the discriminative cue to L-5HTP in rats using the T-maze paradigm (training dose of L-5HTP = 50mg/kg). Numbers in columns indicate hours after lights on.



4.5. Discussion

The most intriguing aspect of this work is the finding that melatonin was unable to evoke an internal state distinguishable from that of saline even at the relatively high dose of 50mg/kg. No difference in the rate of responding or the response latency was detected between saline and melatonin training days which provides additional, albeit circumstantial, evidence that the internal cognitive state of the animals was no more disturbed by melatonin treatment than by saline treatment. The primary site of stimulus control of behaviour by most drugs appears to be of central rather than peripheral origin (Holtzman and Locke, 1988). The behavioural effects of melatonin as described by Gaffori and Van Ree (1985) together with the mounting evidence that the neuroendocrine effects of melatonin are centrally mediated through its interaction with the hypothalamus and the pituitary gland suggest that the compound would be a promising candidate for a drug discrimination study. The failure of melatonin to evoke a discriminative stimulus indicates that this ability of a compound depends on the activation of centres (or receptors) distinct from those that are involved in the neuroendocrine and behavioural effects of melatonin. Moreover, the suggestion by Namboodiri et al. (1983) that, since exogenous 5-HTP elevates plasma melatonin levels, some of the clinical effects of 5-HTP might be mediated by melatonin, does not apply to the discriminative stimulus properties of L-5HTP. Attempts to identify the specific neuroanatomical sites responsible for the discriminability of various centrally-active drugs have met with limited success (e.g. Shannon and Holtzman, 1977; Rosecrans and Glennon, 1979). This is most likely due to cuing properties of drugs representing a compound stimulus derived from a wide spectrum of

drug actions and neurotransmitter interactions.

It is unlikely that a greater number of trials for each session or a greater number of training sessions would have made much difference to the result. The number of trials given to an animal after the first is simply used as a means of reinforcing the required response. It follows that discriminative proficiency prior to the delivery of the first reinforcer must be controlled by the drug-elicitted state, while discriminative proficiency on subsequent trials reflects control by either the drug cue, the cue elicitted by the reinforcer, or a combination of both. In theory this suggests that one-trial training would develop a response that is more drug-orientated than reinforcer-orientated and therefore reflects drug actions more appropriately. In practice, it has been found that although the single-trial training procedure can produce a stable and reliable acquisition, the technique also retards the rate at which a discriminative cue can be acquired (Barry and Krimmer, 1978; Tomie et al., 1987). Tomie et al. (1987) have confirmed that the multiple-trials training procedure leads to a shift in dominance between the drug and reinforcer cues after the first reinforcement rendering all subsequent choice behaviour per session uninterpretable (Tomie et al., 1987). The most common number of trials per session used in drug discrimination studies is between 8 and 10. More than this does not add to the ability of an animal to discriminate a drug-induced cue since by the eighth trial the drug influence has been almost entirely replaced by the cue exerted by the reinforcer.

Increasing the dose of a drug tends to reduce the time to criterion except when toxicity or disruptive behavioural effects emerge to inhibit appropriate responding (Overton, 1982). No change

was observed in the overt behaviour expressed by the animals when given melatonin regardless of dose and training paradigm. It is usual for discrimination to be acquired within 40 sessions (i.e. 20 drug and 20 saline sessions) (Overton, 1982) and this was therefore chosen as a suitable point at which the dose of melatonin could be increased if no sign of cue acquisition was apparent. The training dose can also affect the pharmacological nature of the cue, lower doses are likely to be more specific with respect to the origins of the cue than higher doses (e.g. Colpaert, 1982; Stolerman, 1984). It was therefore considered that, provided a sufficiently high enough dose was chosen, it would be possible to establish a discriminative cue to melatonin based on its hypnotic effects. Since this was not found to be the case the most likely and plausible conclusion is that the final dose used was not great enough to elicit a discriminative stimulus. That sedation was not induced is also supported by the lack of effect on response latency and reponse rate. Several other reasons can also be offered. A drug administered via the i.p. route relies almost entirely on the mesenteric circulation for absorption and would be immediately transported to the liver. Since hepatic metabolism is the major route of peripheral melatonin elimination then a high proportion of the dose can be expected to be metabolized before reaching its site of action. This explanation is unlikely following previous reports concerning hypnotic effects of melatonin by Holmes and Sugden (1982) and Dugovic et al. (1989b), both of which used much lower doses administered i.p. The most simple possibility is that melatonin does not act as a discriminative cue. This might be derived from a rather more speculative interpretation involving closer examination of the hypnotic effects of melatonin. Unlike the barbiturate and

benzodiazepine sedatives/hypnotics melatonin has been reported as being free from "hangover" effects. Melatonin is supposed to elicit sedation by reducing the time to sleep onset and not interfering to any great extent with the regular pattern of alternating slow wave sleep and REM sleep episodes (Cramer et al., 1974; Waldhauser et al., 1990). International travellers have reported a more comfortable transition between time-zones when taking melatonin as opposed to placebo, i.e. their internal state returns to normal more rapidly. Thus failure to evoke a discriminative stimulus might simply reflect the possibility that melatonin, although capable of affecting central processes, does so at a level imperceptible to the animal. Using a method analogous to that chosen by Weissman (1976) to demonstrate the difference in discriminability of aspirin in arthritic rats and non-arthritic rats it might be possible to create a state within an animal that can subsequently be resolved by melatonin and not saline if any melatonin-derived discriminative cue is to be assessed.

The discriminative stimulus induced by L-5HTP is generally thought of as being derived from the activation of 5-HT_1 receptors, though the subclass has yet to be identified due to the lack of specific antagonists for the 5-HT_{1A} and 5-HT_{1B} receptors. Cunningham et al. (1985) suggested that there might also be a role for the 5-HT_2 receptor since although ketanserin does not block the L-5HTP cue directly it is capable of inhibiting the generalization of LSD to L-5HTP. Another interpretation is that this property of ketanserin, rather than reflecting a common origin to the LSD and L-5HTP discriminative cues, is due to the antagonism of 5-HT_2 receptors "upstream" to the 5-HT_1 receptors activated by L-5HTP. It is thought that 5-HT is the actual compound that mediates the L-5HTP

cue since build-up of brain 5-HT correlates with L-5HTP-appropriate responding (Friedman et al., 1983). This would lead to the random activation of 5-HT receptors, whereas the use of LSD results in a more selective action which is more susceptible to antagonism by suitable agents. Such a concept might also explain the failure of melatonin to affect the discriminative cue induced by L-5HTP. The generalization characteristics of 8-OHDPAT and 5MeODMT to the L-5HTP discriminative stimulus are in agreement with the results of Moser and Redfern (1985b) who had previously found that the cue generalizes fully to RU24969 and 5MeODMT. Partial transfer of the stimulus was reported to occur with 8-OHDPAT and TFMPP. These results suggest that the L-5HTP may possess two components, one derived from 5-HT_{1A} receptor activation and one from 5-HT_{1B} activation. This would explain why RU24969, which has approximately equal affinity for these two 5-HT receptor subtypes, is able to demonstrate full generalization to the L-5HTP cue along with the non-selective 5-HT agonist 5MeODMT. 8-OHDPAT is selective for the 5-HT_{1A} receptor, while the action of TFMPP is reported as being selective for 5-HT_{1B} and 5-HT_{1C}, having little or no activity on the 5-HT_{1A} receptor (Kennett and Curzon, 1988b). Thus, from the work of Stolerman (1984) on shared stimulus characteristics as discussed in the introduction, both these compounds can be expected to display partial rather than full generalization to the L-5HTP discriminative cue.

The failure of melatonin to substitute for, or antagonize, the L-5HTP stimulus, supports the evidence presented in the previous chapter that there is little or no interaction between the behavioural aspects of 5-HT_1 receptor activation and melatonin. The development of compounds with greater selectivity towards the

various 5-HT_1 receptor subtypes will allow for a more comprehensive study into possible 5-HT/melatonin interactions using the drug discrimination technique. For example, Tricklebank et al. (1985) demonstrated the discriminative properties produced by 8-OHDPAT were most likely the result of 5-HT_{1A} receptor activation. It is likely that the stimulus induced by TFMPP, the putative 5-HT_{1B}/5-HT_{1C} agonist, is effected by 5-HT_{1B} receptors since no generalization occurs with 8-OHDPAT, but the stimulus does transfer to RU24969, which has no practical affinity for the 5-HT_{1C} receptor. Discriminative studies using 8-OHDPAT and TFMPP as training compounds would allow a more definitive assessment of any potential behavioural interaction that melatonin might have with 5-HT_1 receptor subtypes. The discriminative stimulus properties of the hallucinogenic compounds DOI, DOB and DOM have been attributed to their selective agonist action on the 5-HT_2 receptor (Glennon et al., 1982; Glennon et al., 1983b; Glennon et al., 1984b; Glennon et al., 1986). The results obtained by Dugovic et al. (1988; 1989a, 1989b) suggest that an investigation into whether melatonin can compromise the cue generated by these compounds would be interesting.

In this series of experiments the Skinner box was used to give a food-reward to deprived rats as a reinforcement for the correct response. The T-maze paradigm used escape from a painful stimulus as reinforcement for the correct response. Most drug discrimination studies conform to this arrangement, although some workers have utilized the T-maze as a food-reward procedure (e.g. Barry et al., 1965) and the Skinner box as a shock-escape procedure (e.g. Krimmer, 1974). Since two contingencies have been used in two paradigms direct comparison between the T-maze and the Skinner box

is difficult. However, there are clear methodological advantages of the Skinner box over the T-maze as a means of conducting drug discrimination experiments. The Skinner box yields a greater amount of information concerning the action of an animal and, with the benefit of operant conditioning, offers a greater versatility in the method by which an animal can gain its reinforcement, which can potentially yield more information on the psychoactive properties of the training compound. Automation of the procedure and computer-assisted data-analysis provide additional practical advantages over the T-maze. Actual results obtained from the two paradigms once the animals have been trained are generally compatible but Barry and Krimmer (1978) have found that the food-reward technique as opposed to shock escape can affect the sensitivity of discrimination which could presumably in turn affect acquisition.

An outstanding feature of the data generated by L-5HTP is the high number of sessions required to reach criterion when used as the training drug for the T-maze paradigm in comparison to the Skinner box (53 +/- 3.9 s.e.m. and 20.7 +/- 2.3 s.e.m., respectively). Such a result is not easily explained by a difference in methodology since previous studies have reported similar rates of acquisition for the L-5HTP cue regardless of the paradigm (Barrett et al., 1982; Friedman et al., 1983; Moser and Redfern, 1985b). The most likely explanation is that in the T-maze the rats were trained, essentially, according to a single trial procedure which, as mentioned above, leads to a stable cue but one which is more slowly acquired (Barry and Krimmer, 1978; Tomie et al., 1987). This was brought about by an inherent disadvantage in the wooden construction of the T-maze apparatus used in this series of experiments. Rats

subjected to stress (e.g. an electric shock) commonly, urinate and defaecate. It was realized at the beginning that this posed a potential threat to the integrity of the shock intended for delivery through the steel rods used as a floor for the T-maze. To avoid any possible short-circuiting caused by urine and faecal material deposited on the rods from seeping into the wooden walls after all trials the floor was wiped with dry tissue. This may have proved to be inadequate with the result that certain trials were conducted with reduced effectiveness of the reinforcing cue.

The response rate and response latency are examples of further data provided by the Skinner box and not the T-maze, although an equivalent parameter for response latency could be obtained with the latter paradigm if animals were timed from the moment they were placed into the T-maze to when they entered the safe area. It was found that the early L-5HTP training days greatly affected response latency to the extent that several animals failed to respond at all in their 15 min training session. Persistence of this continued in three animals despite the reduction in L-5HTP dose from 50mg/kg to 35mg/kg and so were withdrawn from the experiments. The response latency parameter on L-5HTP days quickly adopts a similar level to saline days with repeated training. Interestingly, this is not shared by the response rate which remained at a significantly reduced level during L-5HTP training sessions in comparison to saline training periods. The reason for this discrepancy is not clear but it may reflect differing levels of influence for the action of L-5HTP. It is a reasonable assumption that the response latency characteristic represents a quantitive measurement of the decision-making process for the animal. Therefore, the response latency measured in early training sessions

can be expected to reflect the time spent by the rodent pressing the wrong lever before moving to the correct one. As training progresses and the animal becomes confident of making the correct decision the first reinforcement is quickly obtained. This would result in all early training days for all drug treatments having large response latency times which become progressively smaller as training continues, assuming a successful acquisition of the discriminative cue. Fig. 4.9 shows clearly that this was only partially obtained. Animals that received melatonin demonstrated a similar level of response latency on both saline and melatonin training days throughout the experiment. Those rats undergoing training to discriminate saline from L-5HTP expressed a much higher response latency on the early L-5HTP training days than on saline training days. The disruptive effect that L-5HTP possesses on motor behaviour is likely to be responsible for the reduced rate of lever pressing apparent on drug training days - an effect reproduced elsewhere (e.g. Cunningham et al., 1985). This also probably accounts for the high response latency of the early L-5HTP training sessions. However, the development of tolerance to the cognitive effects of the drug may well be responsible for the subsequent reduction in the response latency parameter. Tolerance to the centrally-mediated effects of L-5HTP on motor behaviour has not been reported which would explain the persistence of the reduced response rate. It should be noted that some residual effect on response latency caused by drug-induced disruption of motor behaviour probably persisted throughout the experiment since drug-training days tended to yield higher response latency values than non-drug-training days (see Fig. 4.9). Tolerance looks the most probable reason for the shift in gradient of the dose response curve (see Fig. 4.7) for L-5HTP. It

may be that continued training "fine tunes" the discriminative stimulus, though this observation has not been reported elsewhere with respect to L-5HTP.

The work of Holloway and Wansley (1973a; 1973b; Wansley and Holloway, 1976) suggest that circadian and/or ultradian rhythmic processes may produce state dependent oscillations in retention performance of learned behaviours in animals. Awareness of these studies and others (e.g. Davies et al., 1973) suggest that any circadian fluctuation observed in the discriminative stimulus of any drug may be related to a biological rhythm of performance (in this instance reflecting learning, retention and recall) rather than variability in the degree of strength expressed by the stimulus. Since Moser (1986) had already shown that a group of animals trained at a specific time phase of the LD cycle did not learn the discrimination task any more quickly than another group trained at another time, it was decided to test signal strength variability in pre-trained rodents. Neither of these two methods rule out the performance factor which is difficult to control. The middle and high dose used evinced no appreciable variability in performance/ stimulus strength. At the lowest dose used an apparent reduction in the percentage of correct choices was observed over the ML and MD periods. A more extensive study is required, however, before any significance can be attached to these results.

CHAPTER 5.

MELATONIN AND CIRCADIAN RHYTHMS

5. MELATONIN AND THE CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY

5.1. Introduction

Melatonin may have ambiguous effects with regard to affecting locomotor activity directly but it demonstrates an intriguing ability to modify the expression of the circadian rhythm of locomotor activity. Early studies investigated the pineal gland as a potential biological clock. This concept, first proposed by Wurtman and Axelrod (1965), has been sustained in lower vertabrate classes of animals (e.g. sparrows) but not mammals. Responsibility for circadian time-keeping in mammals appears to have been "taken over" by the SCN and its surrounding structures. Quay (1970a) found that pinealectomy increased the rate of re-entrainment of the locomotor activity rhythm following a phase-shift in the 24 h LD cycle, a property which appears dependent upon the age of the animal (Quay, 1970b, 1972). Conversely, Cheung and McCormack (1982) demonstrated that pinealectomy or continuous melatonin treatment via a subcutaneous silastic implant failed to affect the period of free-running locomotor activity rhythms in dim LL in rats. In addition, they found that this period lengthened when animals were shifted from DD to LL and shortened when shifted back to DD to the same extent in both pinealectomized or non-pinealectomized rats, in keeping with Aschoff's Rule. These results would suggest that despite the importance of the LD cycle on the function of the pineal gland the integrity of the gland is not important in the generation and maintenance of mammalian circadian activity rhythms. Since this time, however, it has been shown that melatonin entrains free-running locomotor activity rhythms but only if given as a pulse during a narrow window of sensitivity in the cycle. This sensitive

phase corresponds to the end of the subjective day when diurnally-active animals enter their quiescent period and nocturnal animals become active (Redman et al., 1983; Gwinner and Benzinger, 1978). Cassone et al. (1986a, 1986b) confirmed and extended the results of Redman et al., identifying that this reaction to melatonin requires an intact SCN and is dose-dependent. To a limited extent melatonin is capable of re-establishing disrupted rhythms induced by gradual lengthening of the photoperiod to LL conditions but fails to entrain those animals whose free-running activity rhythms remain intact in LL (Chesworth et al., 1987).

It is interesting to note that the failure of continuous melatonin release to affect free-running activity rhythms compares with the ability of similar treatment to inhibit pulsatile melatonin-induced gonadal regression (Goldman et al., 1979). Additionally, Armstrong and Redman (1985) have reported that the period of activity in rats maintained under free-running (DD) conditions is extended if melatonin is administered in the drinking water. This is not quite the same, though, as continuous therapy since rats drink mostly during their active phase and no entrainment of their locomotor activity rhythm was detected. Melatonin would appear to execute entrainment by inducing phase-advances between CT10 and CT11 (circadian time 10 and 11, respectively, where CT12 corresponds to the start of the active phase)(Armstrong, 1989). There remains some doubt as to whether this is the correct interpretation since a number of vehicle-treated animals displayed small phase-advances when injected at CT10. It would appear that this phase point in the activity cycle is particularly sensitive to potential zeitgeber irrespective of whether they are internally or externally derived. The phase response curve (PRC) is a plot that

indicates how the extent and direction of a phase-shift induced by a single stimulus depends on the phase at which the stimulus is applied. PRCs generally have phase-advance and phase-delay components. The mammalian PRC for melatonin is unusual in that it is characterized almost entirely by phase advances which occur at a time when light pulses would induce phase-delays and is devoid of activity in this respect at other times of day (Armstrong, 1989), although this does ignore two anomalous results reported by Armstrong in the same article where melatonin produced a 50 min phase advance in one rat at CT22 and a 20 min phase-delay in another animal at CT18. The influence of melatonin as an internal zeitgeber for locomotor activity in lower classes of vertabrates has been less controversial; e.g. Underwood (1986) has described a full PRC which possesses phase-advance and phase-delay components for melatonin in lizards; continuous melatonin therapy can lengthen the period in certain species of lizard (Underwood, 1979, 1981); and it has been reported that continuous melatonin reduces the period of activity of sparrows in both free-running and entrained conditions (Turek et al., 1976b; Hendel and Turek, 1978). The action of melatonin on the activity rhythm of mammals under entrained conditions has yielded more practical results. A recent study has found that small doses of melatonin given to Djungarian hamsters housed under a long photoperiod regime (16:8 LD cycle) 12 h after the onset of the light phase induces a short-day response in activity in receptive animals (Puchalski and Lynch, 1988). That is, in conjunction with the physiological adjustments such as gonadal regression, moulting and body weight loss associated with short-days, the period of locomotor activity was advanced and its duration extended. Armstrong (1989) has presented data that suggests melatonin may phase advance the

onset of locomotor activity in the rat if given during the late afternoon before the onset of darkness in a 12:12LD cycle provided there is not a phase angle difference between activity onset and dark onset greater than 3 h (45°), though this has not yet been reproduced elsewhere.

The internal synchronizing properties of melatonin on the biological clock have lent credence to the concept of melatonin as a "jet-lag alleviator". Early studies describing the effects of pinealectomy and melatonin on the rate of re-entrainment after a phase-shift of the LD cycle were less promising. Pinealectomized animals re-entrained at a faster rate than controls (Kincl et al., 1970) and Quay (1972) showed that this rate reduced with age. Interestingly, Benus et al. (1988) has recently found that aggression and rate of re-entrainment are connected. Wild house mice selected for their high aggression levels entrained to a 12 h phase-shift in the LD cycle less quickly than mice selected for their low levels of aggression. Benus et al. have proposed that aggressive mice have stronger links with their endogenous pacemakers making them more resistant to changes in environmental time-cues. In a social context these results suggest that subordinate animals would be forced to live to the vagaries of the dominant group member and entrain to new environmental conditions at the rate determined by the behaviour of the dominant member. The impact of social interactions on entrainment has been considered in greater detail by Mrosovsky (see Mrosovsky and Salmon, 1987; Mrosovsky, 1988).

Treatment with melatonin itself has provided ambigous data in the study of synchronizing rhythms to a new LD cycle. If removal of the pineal gland and therefore most of the circulating melatonin increases the rate of re-entrainment, one might expect

melatonin treatment to decrease it. However, Murakami et al. (1983) reported that a melatonin pellet implanted near the SCN of intact and pinealectomized rats accelerated re-entrainment of the circadian rhythm of blood corticosterone after inversion of the LD cycle. Redman and Armstrong (1988) were unable to provide consistent data in support of their claims that melatonin could affect the rate and direction of re-entrainment according to the time of injection relative to the original or intended LD cycle. However, Arendt and co-workers have produced persuasive evidence in support of a role for melatonin alleviating the symptoms of jet-lag (Arendt et al., 1987). Human subjects were transported across eight time-zones in an eastward direction which required an 8 h advance of their circadian rhythms for synchronization to the new time period. An oral dose of melatonin (5mg) or placebo was taken for three days prior to and on the day of travel at 18:00 h according to their current time zone. This would correspond to the latter half of their active phase. Treatment was continued for four days after arrival at their destination but the dose was taken between 22:00 h and 24:00 h according to their new time zone. This would approximately correspond to the end of their newly-adjusted active period, which is consistent with studies conducted by Gwinner (Gwinner and Benzinger, 1978) in diurnally active starlings and Underwood (1986) using lizards as being the most appropriate time to entrain free-running locomotor activity rhythms of a diurnally active species using a bolus dose of melatonin. Melatonin-treated subjects suffered significantly less symptoms of jet-lag as determined by a subjective analysis using a visual analogue scale and were reported as being less depressed and more alert than placebo-treated subjects. Additionally, urinary analysis of endogenous melatonin and

cortisol rhythms demonstrated a more rapid resynchronization in melatonin-treated subjects than controls (Arendt et al., 1987; Arendt, 1988). The beneficial effects of melatonin in the prevention of jet-lag were repeated in a larger double-blind cross-over trial among 61 subjects travelling from Britain to Australia and back.

It was decided to investigate further the potential of melatonin as a co-ordinator of circadian rhythms using long-term monitoring of locomotor activity since it effectively presents a window to the internal circadian state of an organism and, due to the ease by which it can be recorded, is the most commonly used method of analyzing circadian rhythms and their manipulation. The use of a 180° phase-shift in the LD cycle to investigate the effect of a particular treatment on the rate of re-entrainment to the new cycle has been criticized with the argument that animals may phase-advance or phase-delay to re-establish their internal rhythms to the time cues provided by the external environment (Armstrong and Redman, 1985). This procedure was adopted, however, as a means of causing temporary rhythm disruption to the organism with the intention of analyzing the rate at which melatonin may restore synchrony irrespective of the direction of re-entrainment. It was also considered that melatonin might influence the direction of re-entrainment after a 12 h environmentally-induced disruption of circadian rhythms - which, if apparent, could dictate future experiments.

The results published by Redman et al. in 1983 concerning the entrainment of a free-running rhythm by melatonin were intriguing on their own and their reproducibility was considered important. Moreover, stress, induced by the presence of a laboratory worker in the vicinity of the animals at regular time-points in

addition to the stress induced by injection, might be sufficient to entrain free-running rhythms rather than treatment with melatonin. Hence a further series of experiments were conducted to verify the work of Redman et al. Four animals were monitored using the Animex system of locomotor activity analysis while the other group of six animals were monitored using light beams. Both systems relied upon the BBC Model B computer for data collection and the series of programs developed by Marshall et al. (1985) and Mitchell (1989) for data analysis.

A third series of experiments was initiated with the intention of determining the effect of melatonin on the phase-shift of a free-running locomotor activity rhythm induced by a 15 min light pulse. The PRC generated by administering light pulses at different time points of the activity-rest cycle in constant (free-running) conditions has three components. If the pulse is given during the early subjective night, or at the end of the subjective day, when the animal just becomes active (i.e. at CT12) the rhythm is phase-delayed, that is, the beginning of the next activity period is delayed with respect to the expected time of commencement. If the pulse is given during the late subjective night a phase-advance is observed, that is, the inchoation of the activity phase occurs earlier than expected. The maximum degree of phase-shift of the onset of activity from the anticipated time point if no pulse had been administered might take several cycles to become apparent. The third component of the PRC is the non-responsive phase. A light pulse given between approximately CT3 and CT8, the subjective day, or rest phase, for a nocturnally active mammal, elicits no phase-shift in either direction. The overall shape of the PRC generated by light pulses is dependent on the

duration of the pulse and the species under investigation (Honma et al., 1978; Turek, 1987: Daan and Pittendrigh, 1976) The hypothesis was that if melatonin provided the signal of darkness to the internal environment of the organism, as demonstrated by the work of Gwinner and Benzinger (1978) and Redman et al. (1983), a bolus dose of melatonin might counter the effect of a 15 min light pulse. Time prevented the testing of this hypothesis over a range of phase points in the circadian cycle. Examination of past papers revealed that the phase-delay induced by a 15 min light pulse at CT12 might be the most convenient direction of shift to investigate in consideration of the extent of shift and the immediacy of the response (Honma et al., 1978) and it was also considered the most likely period for melatonin to elicit an effect.

The ambiguous results generated by previous studies of the effects of melatonin on locomotor activity prompted an additional investigation into this behaviour. Two types of locomotor activity were analysed. Exploratory locomotor activity (ELA), defined as that level of activity occurring within the first 10 min of an animal being placed into a novel environment, was measured at four time points (ML, EOL, MD and EOD). Spontaneous locomotor activity (SLA) was assumed to be the level of activity occuring in the course of 2 h after an equal period of acclimatization to a novel environment. Spontaneous activity was measured at two time points, ML and MD. The effect of melatonin on activity after a 6 h phase advance of the light or the dark period was also investigated.

5.2. Materials and Methods

5.2.1. Animals

Male Wistar rats (Animal House, University of Bath strain) weighing between 150 and 200g at the start were used in all long-term locomotor activity experiments. Animals were housed individually in standard plastic cages measuring 520x350x170mm with food and water available *ad libitum*. Animals weighing between 250 and 350g were used for the short-term analysis of exploratory and spontaneous locomotor activity. They were housed in standard plastic cages in groups of 4-6 until the time of the experiment which was conducted using individual animals. Food (Labsure CRM diet) and water was available *ad libitum*. Since rats were removed from a colony familiarized to a 14:10 LD schedule, all animals were maintained under a 12:12 LD cycle for at least two weeks prior to use, unless experimental conditions required an alternative lighting regimen.

5.2.2. Drugs

Melatonin (Sigma) up to doses of 1mg/kg was dissolved in normal saline by sonication and larger doses suspended in 0.1% w/v tragacanth in normal saline, also by sonication. All injections were administered i.p. using a dose volume of 1ml per 200g. Control animals received the same volume of vehicle.

5.2.3. Apparatus

Long-term locomotor activity experiments were conducted with locomotor activity cages housed in the environmental cabinet first described by Hillier et al. (1973). These units, as depicted in Fig. 5.1, were constructed from either 17mm blockboard with the dimensions 610 x 450 x 615mm. Animals were further insulated from

external events by 12mm polystyrene sheets covering the walls and door, and draft-excluder tape lining the door frame. Ventilation was provided by an extractor fan (Philips, Type HR3408) feeding 2-3 cabinets via 30mm-bore plastic tubing connected to the rear of a cabinet. A second plastic tube attached to the side of the units allowed passage of a flexible pipe to the interior for the provision of water from a reservoir located above the cabinet if required. Internal lighting, achieved using a 12 inch 8W white-light fluorescent tube (Thorn EMI, Type FLJ 1008) attached to the ceiling of a cabinet provided an illumination intensity of between 650 and 850 lux (Macam Photometrics Ltd., Model Radiometer/Photometer R101) at cage-floor height and was controlled by an externally mounted time switch (Sangamo, Type 524-I-171 or Smith Industries, Type TS500). The choke was also mounted externally on a metal heat-sink to prevent heating the internal environment. VARIMEX activity monitors were used for recording the circadian rhythm of locomotor activity during experiments involving the effect melatonin on the rate of re-entrainment and the first entrainment of free-running rhythms experiment. Animals could be left in these cages for three weeks before the sawdust had to be replaced. This procedure was carried out during the active phase of the rat in the experimental room under dim red light (6 lux). The second entrainment experiment used photocells located in the walls of standard grid-based plastic cages (320 x 500 x 165mm), 40mm above the floor and mid-way between the food-hopper and rear of the cage. The cage was placed on small wooden platforms to allow easy access to the sawdust tray. Some modification to the interior of the environmental cabinet was instigated for the phase-shift experiments and is shown in Fig. 5.2. Four wooden square pillars, 32mm x 32mm x 420mm, two on either

side spaced 300mm apart were used to support four wooden rails (17 x 10 x 510mm). These were used to mount metal grids (with 10mm apertures) using plastic ties. This allowed the photodetectors and photoemitters to be mounted opposite each other outside the activity cage with a high degree of manoeuvrability. This modification necessitated a smaller activity cage (130 x 250 x 420mm) which was placed on a wooden platform across the lower rails. The lid to the cage was raised permitting a full activity cage height of 205mm. Two 0.5 inch diameter holes were drilled into opposite sides of the activity cage 40mm from the base and 210mm from the rear of the cage to permit the passage of the light beam. Rats were prevented from chewing the vulnurable plastic area surrounding the holes by attaching two aluminium plates with 0.5 inch holes in their centre to the interior walls of the cage. Since the cage had a solid plastic floor it was found necessary to replenish sawdust once every two weeks during an experiment. This was performed in the experimental room under dim red light (6 lux). In each experiment sufficient laboratory chow was provided to accomodate the feeding requirements of a single rat for 7 days. Water was dispensed from a standard water bottle and checked every other day. Care was taken to refill water bottles and food, if required, at irregular times of the day.

A second type of environmental cabinet, described by Mitchell (1989), contained the activity monitor used for the short-term analysis of locomotor activity (see Fig. 5.3). The chamber was constructed from 17mm plywood with dimensions 1300 x 900 x 1400mm. Penetration of light and sound were minimized by lining the walls and door with 12mm polystyrene sheets and the door frame with draft-excluder tape. Ventilation was provided by a single

extractor fan (Philips, Type HR3408) connected to the rear of the chamber via 30mm-bore plastic tubes. Internal lighting (100 lux) was achieved using a wall-mounted 12 inch 8W white light fluorescent tube (Osram), controlled by an externally-mounted time switch (Smith Industries, Type TS500). The choke was located outside of the chamber on a metal heat-sink to prevent heating the internal environment. The door to this cabinet was kept open during the analysis of exploratory locomotor activity and closed during spontaneous locomotor activity experiments. The locomotor activity monitor, as described by Mitchell (1989), was constructed from 5mm black perspex with the dimensions 900 x 600 x 350mm (see Fig. 5.4). Three infrared photoemitters and three photodetectors were located at 300mm intervals along the walls of the cage, 50mm above a stainless steel metal-grid floor. A removable perspex tray placed beneath the grid floor was used to hold sawdust. The lid was constructed from clear perspex and housed two descending pillars, 120 x 120 x 300mm, with their respective centres 340mm apart. These contained the partner photoemitters and photodetectors to those located in the walls of the cage such that the infrared light beams were positioned to divide the cage floor area into six equal parts. An additional photoemitter and photodetector were placed in the facing sides of the pillars 50mm above the cage floor. Each photocell was connected to a D-socket mounted in the wall of the environmental cabinet. A food hopper and water bottle were attached to the side of the activity monitor for the provision of nourishment *ad libitum*.

Fig. 5.1. Diagram showing the main features of the environmental cabinet used in the circadian locomotor activity studies.

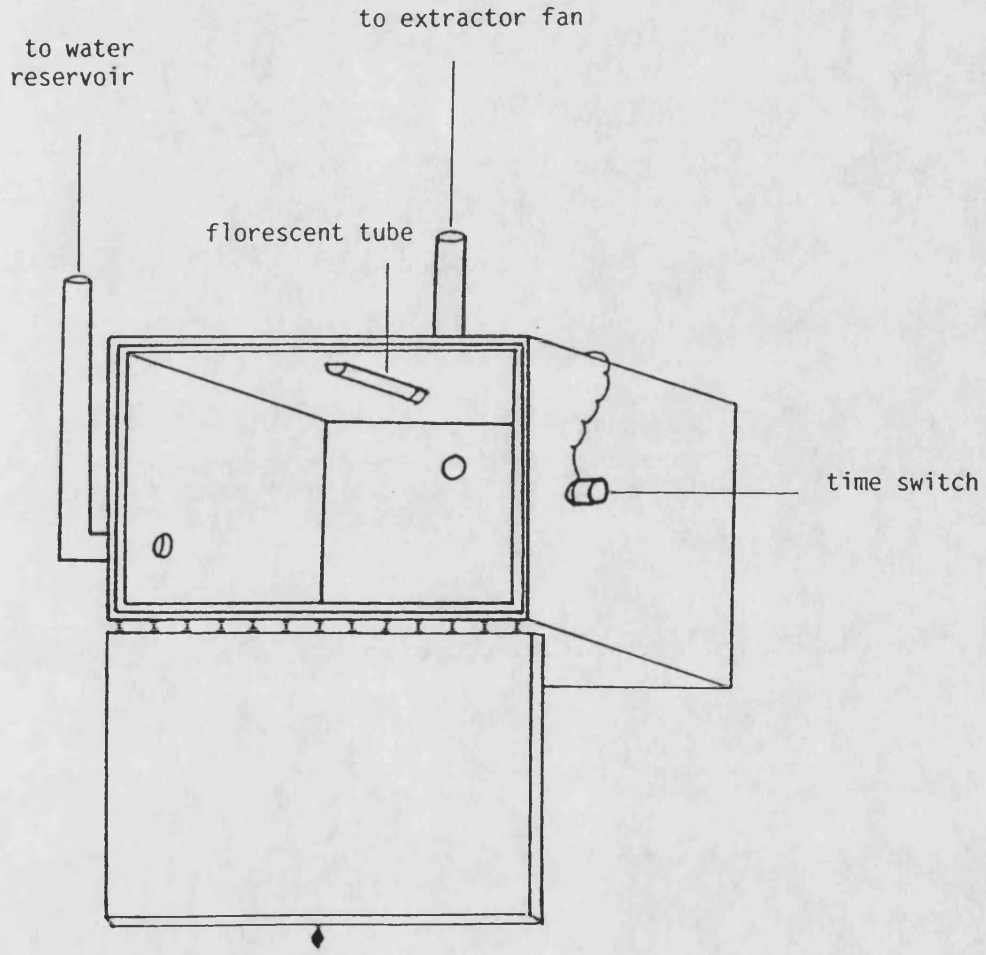


Fig. 5.2. Diagram of apparatus used to hold photocells for phase-shift experiment.

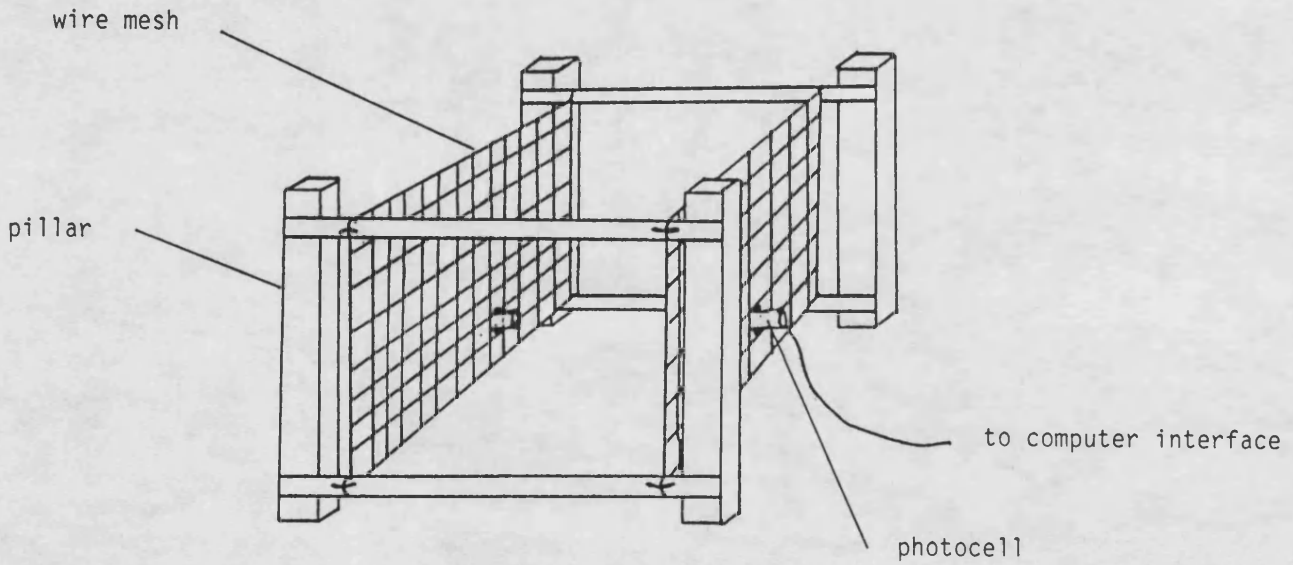


Fig. 5.3. Diagram showing the main features of the environmental cabinet used in the spontaneous and exploratory locomotor activity of individual rats.

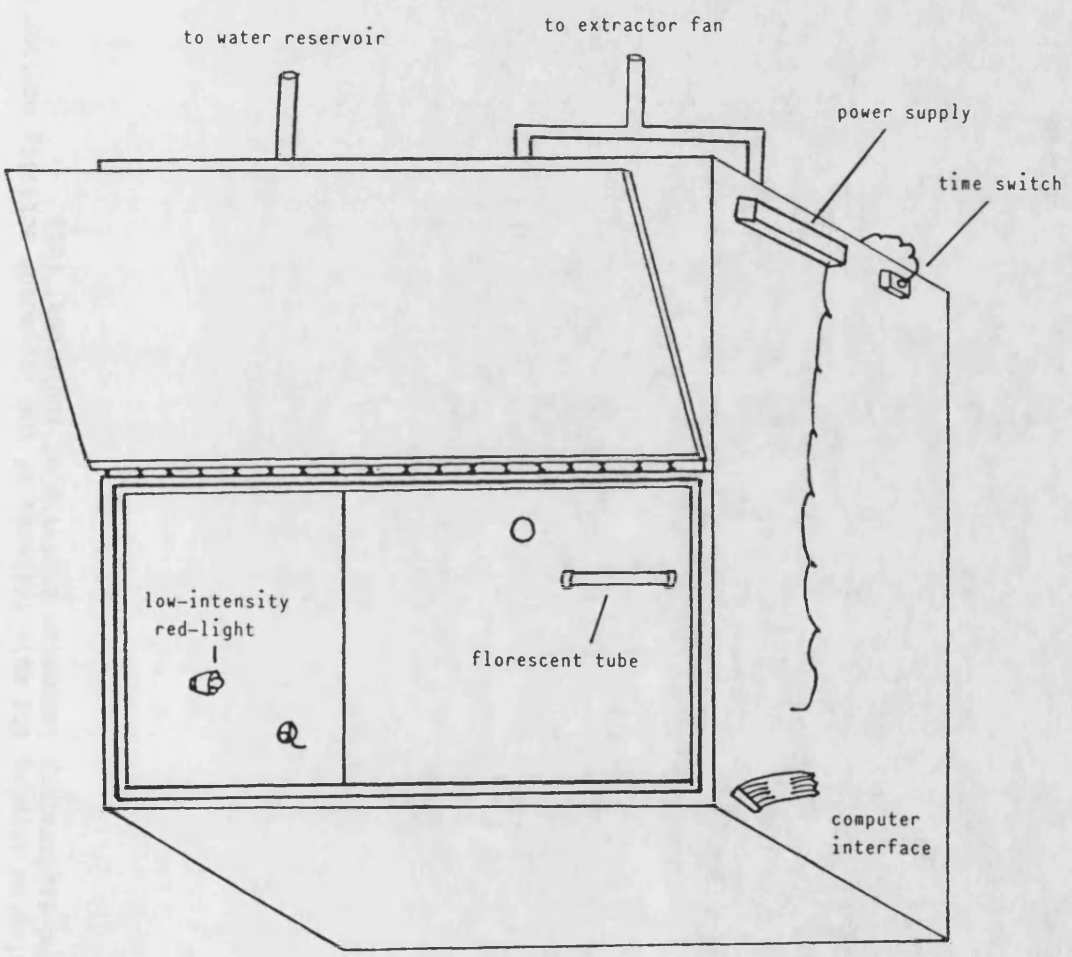
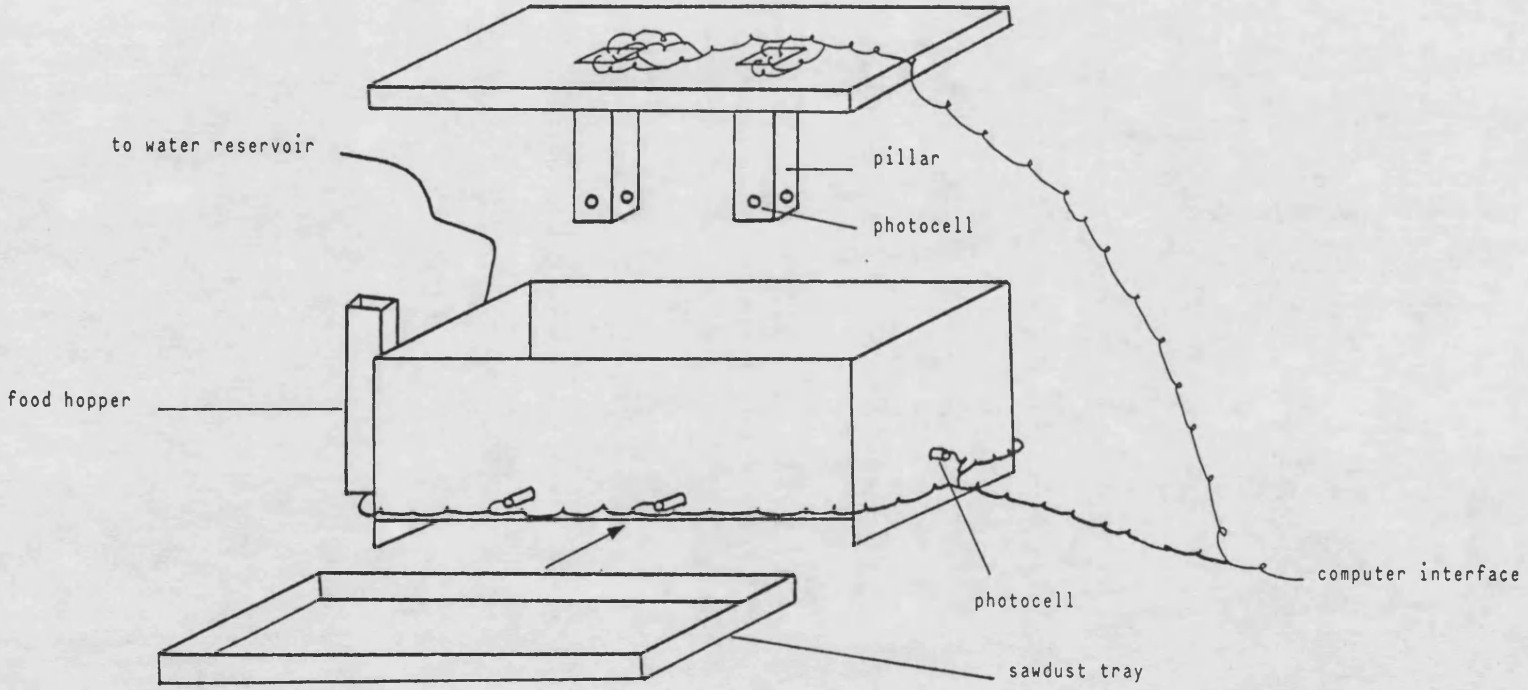


Fig. 5.4. Diagram showing the main features of the locomotor activity monitor used to record spontaneous and exploratory locomotor activity of individual rats.



5.2.4. Data Collection

Two methods of recording locomotor activity were employed in this series of experiments - ANIMEX and Photocells - both of which communicated with a computer (Acorn BBC, Model B) via an interface and software designed by R. Marshall, University of Wales College of Medicine, Cardiff (Marshall et al., 1985), thereby enabling the storage of data on 5.25 inch floppy discs.

The ANIMEX system consisted of a VARIMEX horizontal activity electromagnetic sensor (Columbus Instruments Ltd.) placed in the environmental cabinets beneath the plastic animal cages. A signal from the sensors was recorded by an activity counter (Columbus Instruments Ltd.) and registered as a count on the computer.

The photocell system consisted of two types of photocell per animal cage. The photoemitters (Scan-A-matic, Model L33007) were energized by a 5 volt DC power supply (Radio Spares switched-mode 70W). The response of photodetectors (Scan-A-Matic, Model P33001) to a breakage of an infrared light beam was registered as a count on the computer. Counts were collected for a predetermined time before storage on floppy disc.

5.3. Data Analysis

Long-term locomotor activity experiments generate vast quantities of numbers which represent the level of activity occurring over a predetermined sampling interval. The actual numbers are of secondary importance. Accurate analysis depends on the recognition of trends and patterns derived from the variation in their magnitude. The actogram is the most common means of displaying long-term circadian locomotor activity data and represents the

distribution of activity over a 24 h period. Activity recorded at a level greater than a predetermined threshold is registered as a solid block of width equivalent to the sampling interval printed at its corresponding time. For greater clarity actograms are double-plotted (e.g. see Fig. 5.5a). The main weakness of this method of data presentation is the bias introduced by the experimenter resulting from their choice in threshold. This source of influence was avoided by using the calculated daily median of activity as the threshold. Thus for each 24 h cycle plotted on the actogram there is an independently chosen threshold. Actograms were used in combination with daily activity plots to estimate the onset of activity periods. Assessing the onset of activity in both the rate of entrainment and free-running rhythm entrainment experiments was achieved by the visual inspection of actogram data and, where possible, visual inspection of daily (24 h) activity profiles (e.g. see Figs. 5.7a-5.8b).

A more complicated mathematical technique applied to circadian locomotor activity data is that of periodogram analysis, which is used to determine the period expressed by a rhythm. Periodogram analysis comes under the more general term of "time series analysis" which is used to characterize time series data (i.e. observations made sequentially in time) mathematically. The reader is referred to Chatfield (1985) and De Prins et al. (1986) for background reading and the applications of time series analysis to chronobiology. Periodogram analysis was used to determine the period of the circadian rhythms in locomotor activity. Periodogram profiles were found to be of poor and inadequate resolution if they had been calculated on the basis of less than 8 days data. This is due to accuracy in the determination of the primary period of a

rhythm being dependent on little change occurring over the interval under analysis. Thus this technique was used for determining the period of the locomotor rhythms in experiment 2 where treatment was continued for 14 days and a suitable number of days before and after treatment could also be analysed.

Software for the generation of median determined actograms and periodogram analysis were kindly provided by P. Mitchell (1989).

5.4. Experimental Protocol

5.4.1. Experiment 1: To determine the effect of melatonin on the rate of re-entrainment of the rat circadian locomotor activity rhythm after a 180° inversion of the LD cycle.

Individual animals were housed in environmental cabinets under a 12:12 LD cycle. In view of the results presented by Redman et al. (1983) melatonin (1mg/kg) was injected for three days prior to the phase-shift at the time point corresponding to the post-phase-shift future end of light phase. The phase-shift itself was conducted after a suitable period of time had elapsed to produce a stable actogram trace. The timing of the phase-shift was designed such that the rats were forced to endure 24 h of darkness before the lights came on again, which was just after their daily dose of melatonin. This could be viewed as a 12 h phase advance of the dark (active) phase or a 12 h delay of the light (rest) phase. (In line with this reasoning 24 h of illumination would be described as a 12 h phase advance of the light (rest) phase or a 12 h delay of the dark phase). Treatment with melatonin was continued at the new end of light phase time point until the end of the experiment. Four animals could be experimented on at one time, two drug-treated and two controls. Details concerning the day and time of the injections

are given with Figs. 5.5a-f.

5.4.2. Experiment 2: To determine the ability of melatonin to entrain free-running rat circadian locomotor activity rhythms.

Individual animals were maintained in environmental cabinets under DD and allowed to free-run until an actogram trace was produced which demonstrated a free-running rhythm of locomotor activity. The onset of activity was predicted by examination of the actogram trace in combination with a program designed to plot the profile of activity over any 24 h period (an example plot can be seen in Fig. 5.7a). Injections of melatonin (1mg/kg) began an hour after the approximated time of activity onset with the anticipation that entrainment would occur within 2-3 days when activity onset coincided with the dose of melatonin. Treatment was continued at the same time for 14 days (i.e. each animal received a total of 14 injections). Following the final injection the animals were maintained under DD and left undisturbed (except for necessary hygiene and nourishment requirements) for a further period of time to re-establish free-running activity rhythms. Four animals were monitored using the ANIMEX system of activity analysis (two drug, two controls; rats 1-4) and a further six animals were monitored using photocells (three drug, three controls; rats 5-10). Details concerning the days and times of injection are given with Figs. 5.9a-j.

5.4.3. Experiment 3: To determine the effect of melatonin on the ability of light to elicit a phase-shift in rats.

Animals were housed in modified environmental cabinets and activity cages as described above and shown in Figs. 5.1 and 5.2

under DD. This condition was maintained until a free-running rhythm was obtained with sufficient data to allow as accurate an estimation as possible of the onset of activity using the procedure described in Experiment 2. A 15 min pulse of white light (between 650 and 850 lux) was then administered at the expected time of the onset of activity (CT12) the day following its estimation. Details concerning the day and time when a pulse was given to a particular animal are provided with Figs. 5.12a-h. It is regrettable that equipment and data complications prevented further progress with this experiment. The reasons for this are considered in the discussion.

5.4.4. Experiment 4: Effect of melatonin on exploratory locomotor activity (ELA) in the rat.

Single rats were given melatonin (1, 10 and 50mg/kg) or vehicle, 10 or 30 min prior to being put into the activity monitor (as depicted in Fig. 5.4). The total number of counts registered in 10 min was taken as a measure for exploratory locomotor activity and recorded for later analysis. Experiments were conducted at ML, EOL, MD and EOD.

5.4.5. Experiment 5: Effect of melatonin on spontaneous locomotor activity (SLA) in the rat.

Single rats were allowed to familiarize themselves with the activity monitor (as depicted in Fig. 5.4) for 2 h before being injected with melatonin (1, 10 and 50mg/kg) or vehicle. They were then left for a further 2 h and the total number of counts generated in that time taken as a measure of SLA and recorded for later analysis. Experiments were conducted across the ML and MD periods, that is injections were given at ML or MD. The effect of melatonin

on a 6 h advance of the dark phase and a 6 h advance of the light phase was also investigated. Thus, for example a 6 h phase advance of the dark phase was effected by allowing rats to acclimatize to the activity cabinet for 2 h with the lights on upto their ML phase position, they were then removed for injection and then returned to the cabinet with the lights now extinguished. Activity was monitored for the following 2 h. Injections during the dark phase were carried out under dim red light (6 lux).

5.5. Results

5.5.1. Experiment 1: Effect of melatonin on rate of entrainment.

No effect of melatonin on the rate of entrainment was observed. Both control and melatonin-treated rats accomplished re-synchronization to the new LD cycle by small daily phase-delays and took an average of 6.2 (+/- 0.75 s.e.m.) and 6.8 (+/- 0.31 s.e.m.) days respectively. Figs. 5.5a-5.5f depict the actograms pertaining to this experiment. Although there was a noticeable but transient drop in total daily activity apparent following the day of the phase-shift there was no difference between the melatonin-treated and control groups (see Fig. 5.6). Figs. 5.7a-b and 5.8a-b show an example sequence of 24 h activity profiles of single animals starting from two days before the first injection was given, for a melatonin-treated rat and a saline-treated rat, respectively.

Fig. 5.5a. Effect of melatonin on the rate of re-entrainment following a phase inversion of the LD cycle: actograms of the circadian rhythm of locomotor activity expressed by rats 1 (top) and 2 (bottom), melatonin (1mg/kg) and saline treated, respectively.
Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram.

Initial LD cycle: 12:12 (lights on: 09:00 h)

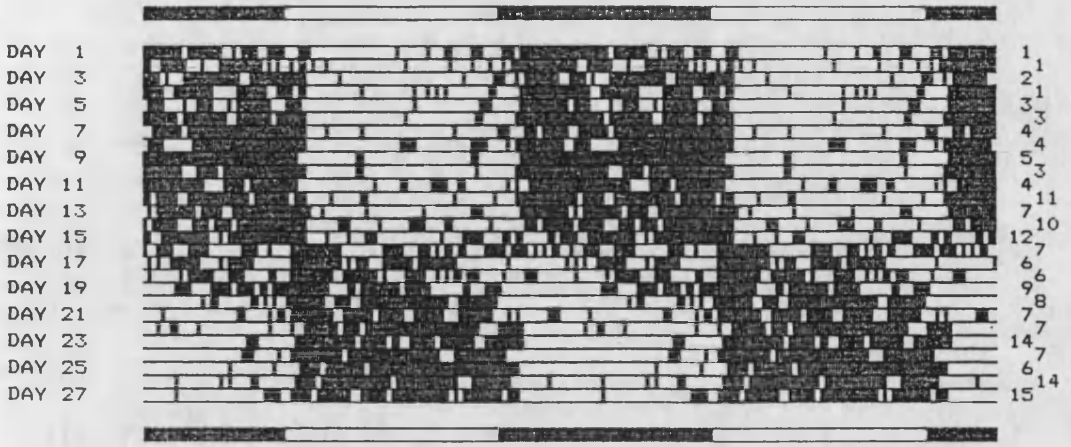
Final LD cycle: 12:12 (lights on: 21:00 h)

Injections begun: day 12 at 08:30 h

Phase inversion: day 15 at 09:00 h (24 h of dark)

ACTOGRAM

BOX 1
THRESHOLD=50% SAMPLING INTERVAL=15Min



ACTOGRAM

BOX 2
THRESHOLD=50% SAMPLING INTERVAL=15Min

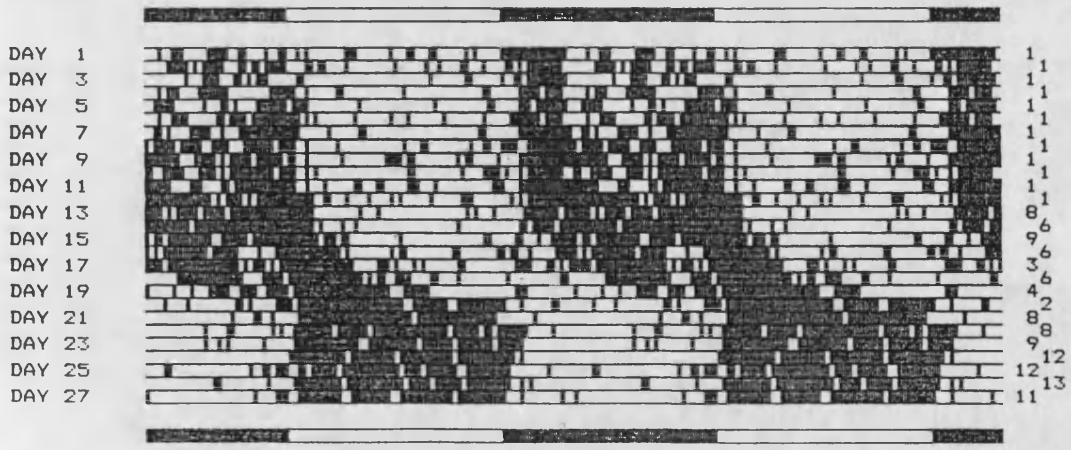


Fig. 5.5b. Effect of melatonin on the rate of re-entrainment following a phase inversion of the LD cycle: actograms of the circadian rhythm of locomotor activity expressed by rats 3 (top) and 4 (bottom), melatonin (1mg/kg) and saline treated, respectively. Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram.

Initial LD cycle: 12:12 (lights on: 09 00 h)

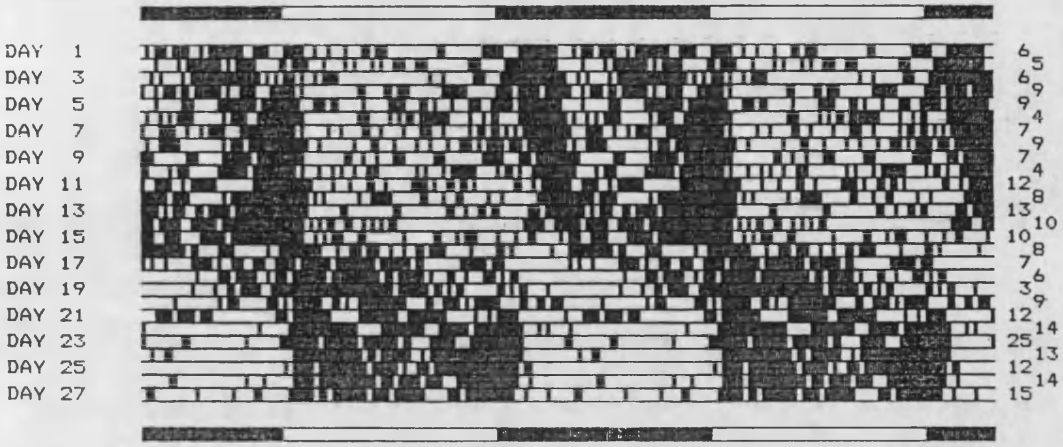
Final LD cycle: 12:12 (lights on: 21:00 h)

Injections begun: day 12 at 08:30 h

Phase inversion: day 15 at 09:00 h (24 h of dark)

ACTOGRAM

BOX 3
THRESHOLD=50% SAMPLING INTERVAL=15Min



ACTOGRAM

BOX 4
THRESHOLD=50% SAMPLING INTERVAL=15Min

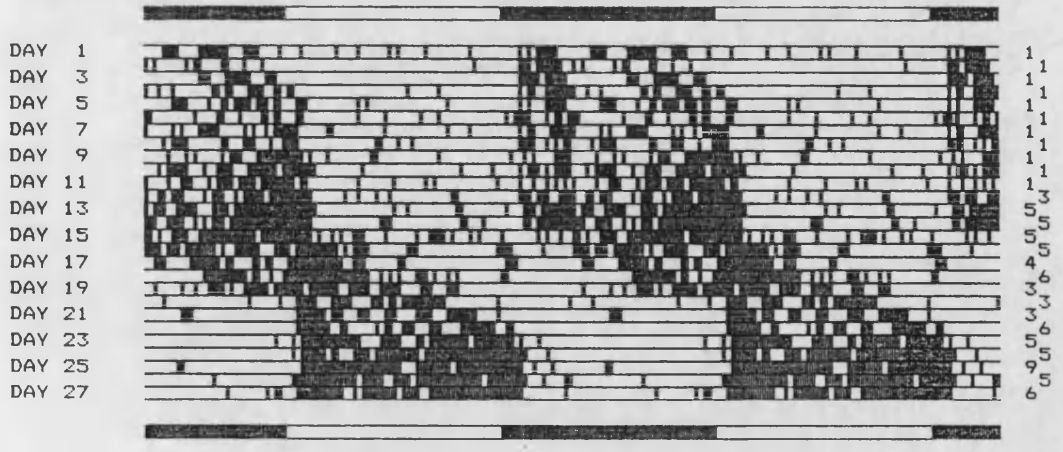


Fig. 5.5c. Effect of melatonin on the rate of re-entrainment following a phase inversion of the LD cycle: actograms of the circadian rhythm of locomotor activity expressed by rats 5 (top) and 6 (bottom), saline and melatonin (1mg/kg) treated, respectively. Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram.

Initial LD cycle: 12:12 (lights on: noon)

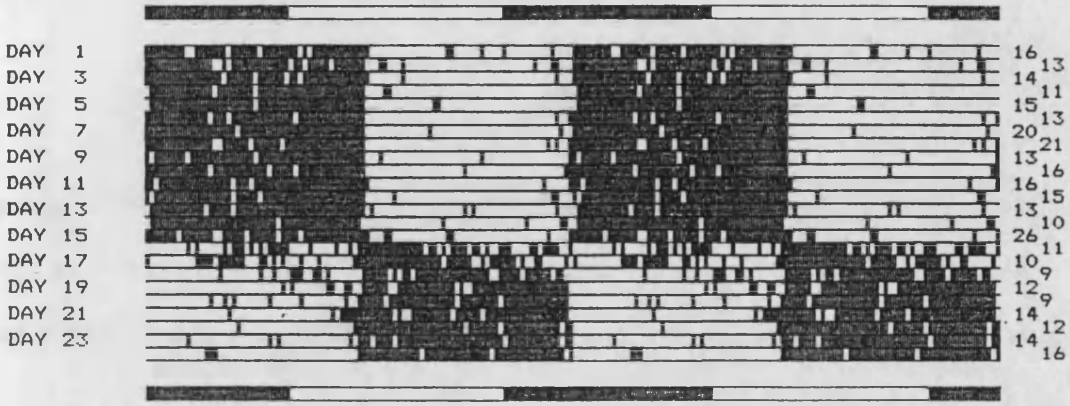
Final LD cycle: 12:12 (lights on: midnight)

Injections begun: day 12 at 11:30 h

Phase inversion: day 15 at noon (24 h of dark)

ACTOGRAM

BOX 1
THRESHOLD=50% SAMPLING INTERVAL=15Min



ACTOGRAM

BOX 2
THRESHOLD=50% SAMPLING INTERVAL=15Min

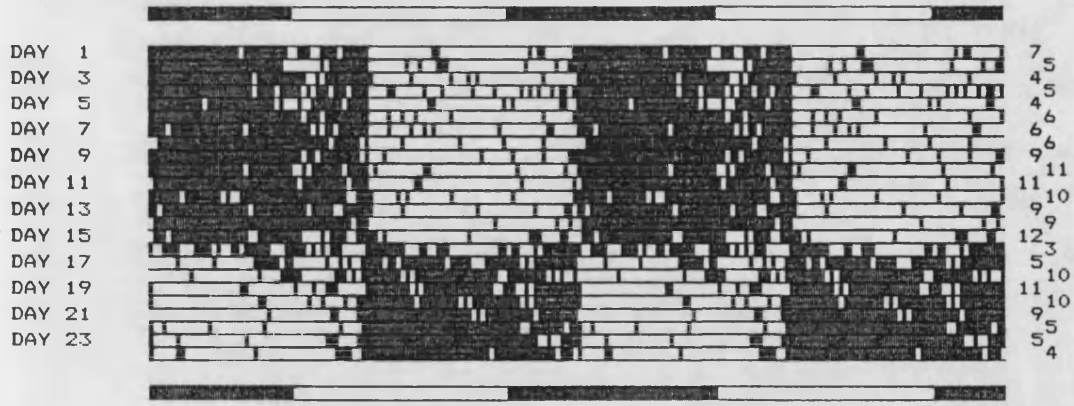


Fig. 5.5d. Effect of melatonin on the rate of re-entrainment following a phase inversion of the LD cycle: actograms of the circadian rhythm of locomotor activity expressed by rats 7 (top) and 8 (bottom), saline and melatonin (1mg/kg) treated, respectively. Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram.

Initial LD cycle: 12:12 (lights on: noon)

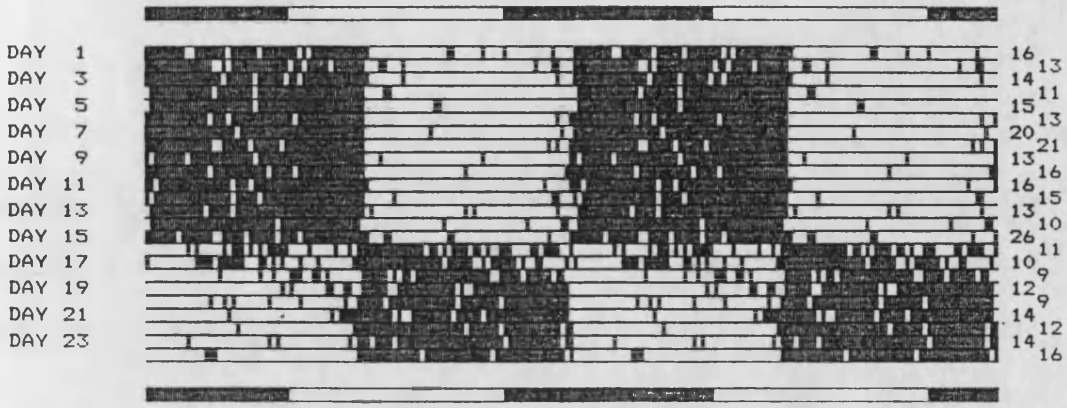
Final LD cycle: 12:12 (lights on: midnight)

Injections begun: day 12 at 11:30 h

Phase inversion: day 15 at noon (24 h of dark)

ACTOGRAM

BOX 1
THRESHOLD=50% SAMPLING INTERVAL=15Min



ACTOGRAM

BOX 2
THRESHOLD=50% SAMPLING INTERVAL=15Min

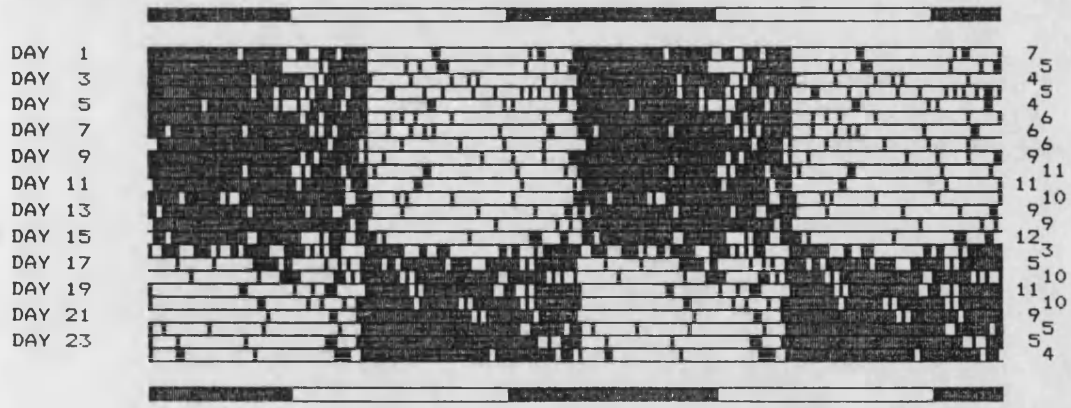


Fig. 5.5d. Effect of melatonin on the rate of re-entrainment following a phase inversion of the LD cycle: actograms of the circadian rhythm of locomotor activity expressed by rats 7 (top) and 8 (bottom), saline and melatonin (1mg/kg) treated, respectively. Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram.

Initial LD cycle: 12:12 (lights on: noon)

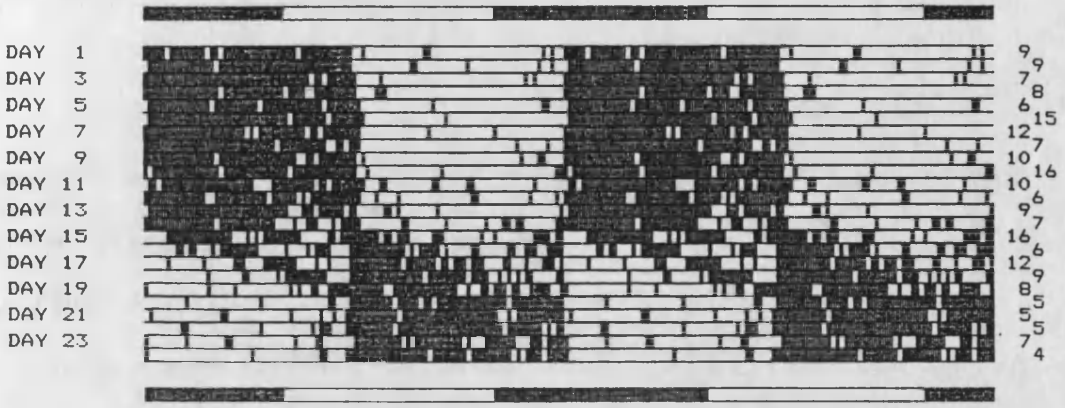
Final LD cycle: 12:12 (lights on: midnight)

Injections begun: day 12 at 11:30 h

Phase inversion: day 15 at noon (24 h of dark)

ACTOGRAM

BOX 3
THRESHOLD=50% SAMPLING INTERVAL=15Min



ACTOGRAM

BOX 4
THRESHOLD=50% SAMPLING INTERVAL=15Min

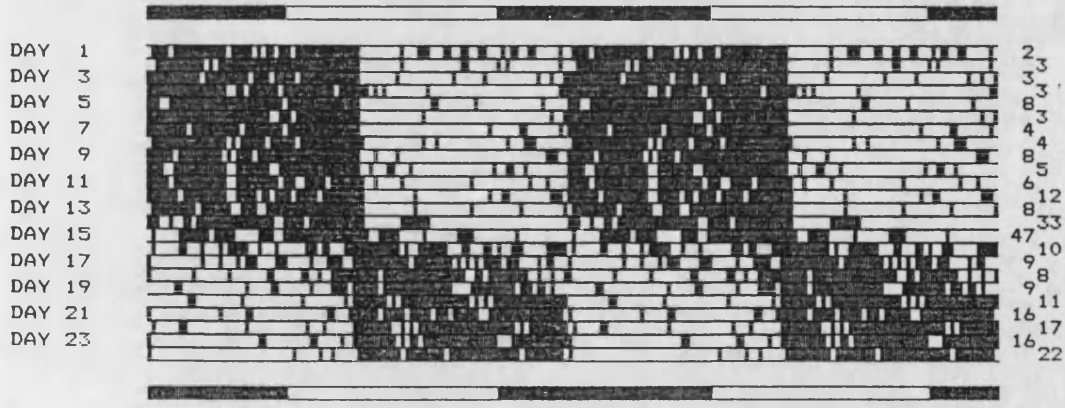


Fig. 5.5e. Effect of melatonin on the rate of re-entrainment following a phase inversion of the LD cycle: actograms of the circadian rhythm of locomotor activity expressed by rats 9 (top) and 10 (bottom), melatonin (1mg/kg) and saline treated, respectively. Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram.

Initial LD cycle: 12:12 (lights on: noon)

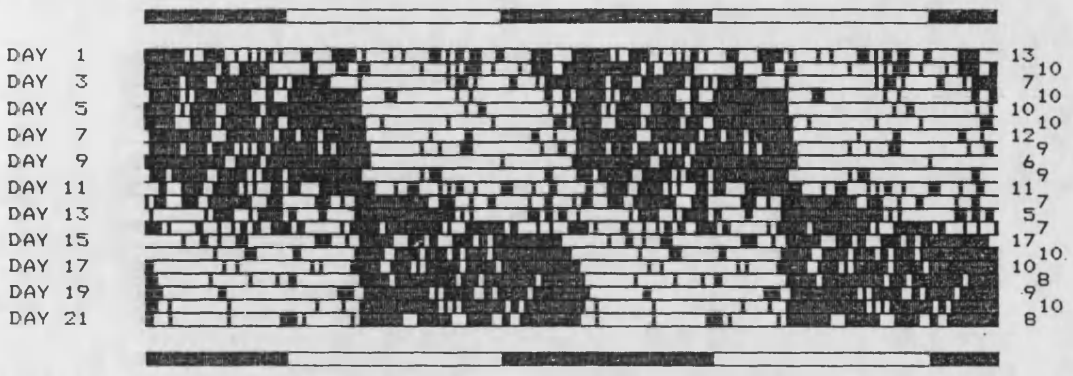
Final LD cycle: 12:12 (lights on: midnight)

Injections begun: day 8 at 11:30 h

Phase inversion: day 11 at noon (24 h of dark)

ACTOGRAM

BOX 1
THRESHOLD=50% SAMPLING INTERVAL=15Min



ACTOGRAM

BOX 2
THRESHOLD=50% SAMPLING INTERVAL=15Min

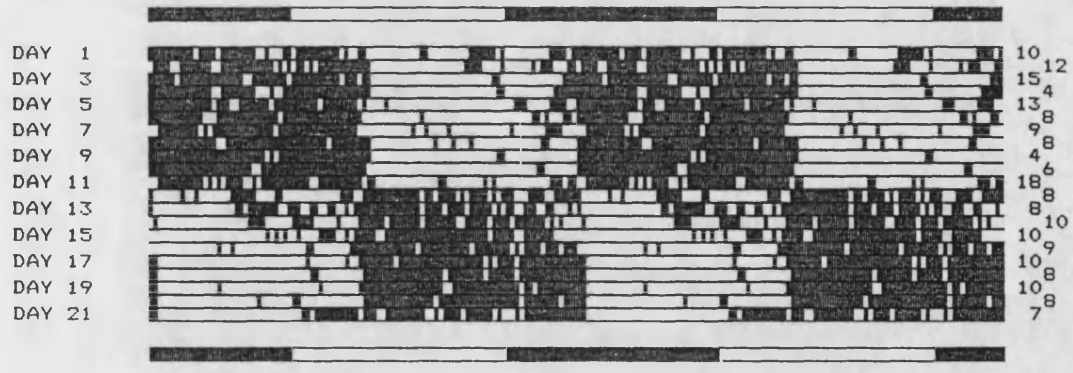


Fig. 5.5f. Effect of melatonin on the rate of re-entrainment following a phase inversion of the LD cycle: actograms of the circadian rhythm of locomotor activity expressed by rats 11 (top) and 12 (bottom), melatonin (1mg/kg) and saline treated, respectively.

Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram.

Initial LD cycle: 12:12 (lights on: noon)

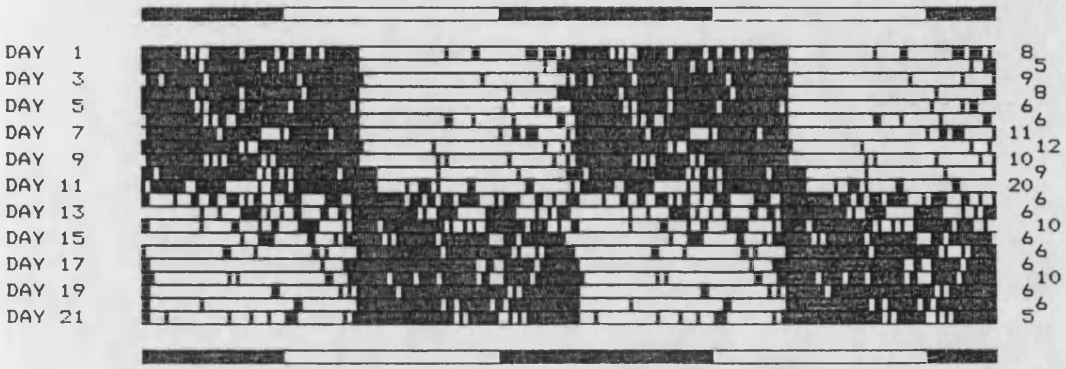
Final LD cycle: 12:12 (lights on: midnight)

Injections begun: day 8 at 11:30 h

Phase inversion: day 11 at noon (24 h of dark)

ACTOGRAM

BOX 3
THRESHOLD=50% SAMPLING INTERVAL=15Min



ACTOGRAM

BOX 4
THRESHOLD=50% SAMPLING INTERVAL=15Min

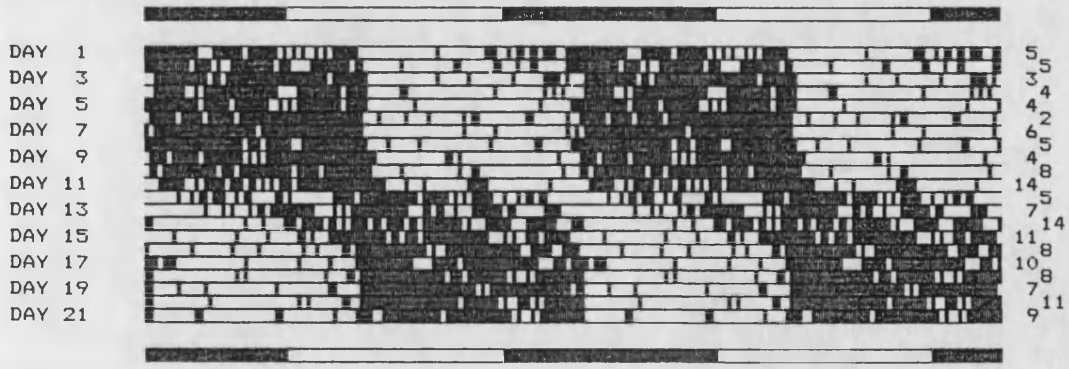


Fig. 5.6. Rate of entrainment experiment - effect of melatonin (1mg/kg) on mean total daily activity in rats before and after a 180° phase-shift of the LD cycle. (○ = saline, ● = melatonin; n=6).

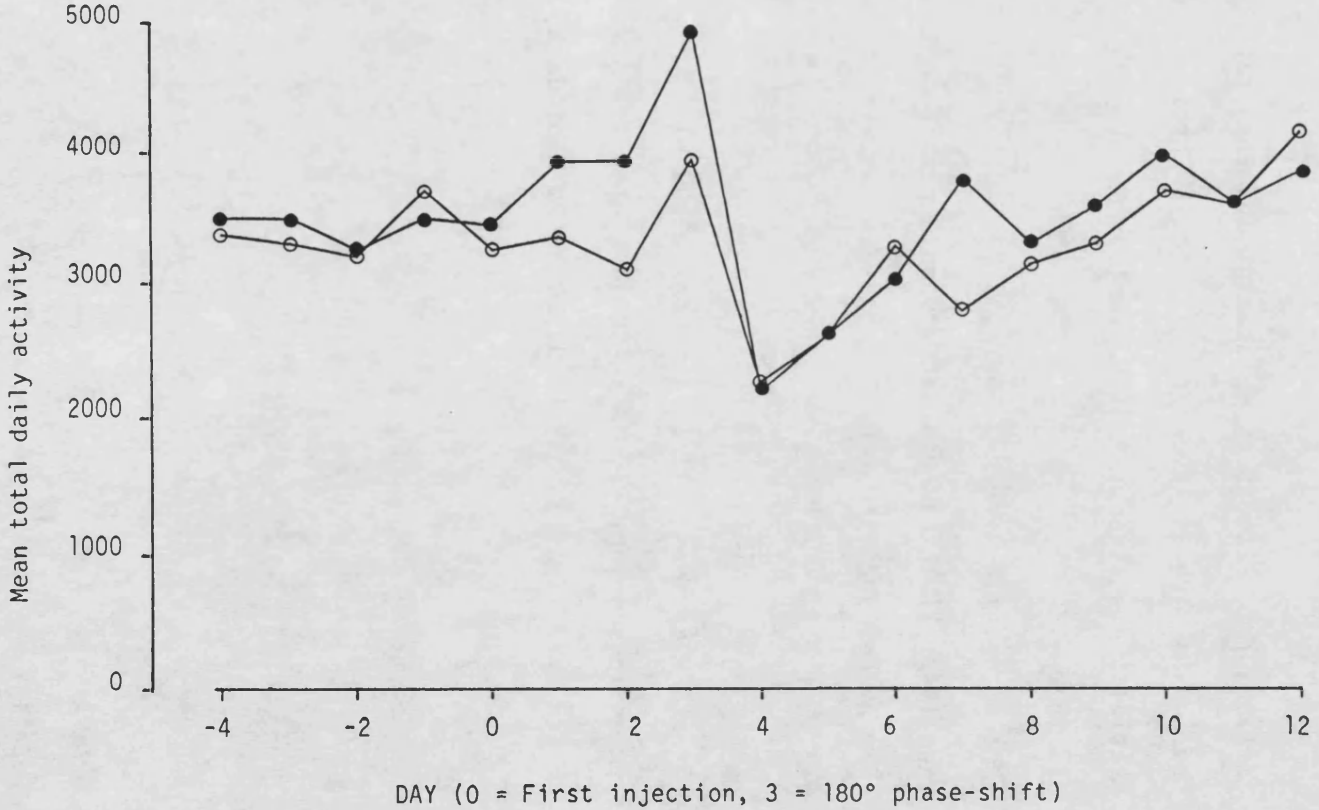


Fig. 5.7a. Effect of melatonin on the rate of re-entrainment following a phase inversion of the LD cycle: sequence of locomotor activity profiles over 24 h from day 10 to 17 for rat treated with saline.

For actogram, see Fig 5.5b.

Abscissa: Time (h). Ordinate: Counts per epoch (15 min).

Day number depicted beneath abscissa.

Initial LD cycle: 12:12 (lights on: 09:00 h)

Final LD cycle: 12:12 (lights on: 21:00 h)

Injections begun: day 12 at 08:30 h

Phase inversion: day 15 at 09:00 h (24 h of dark)

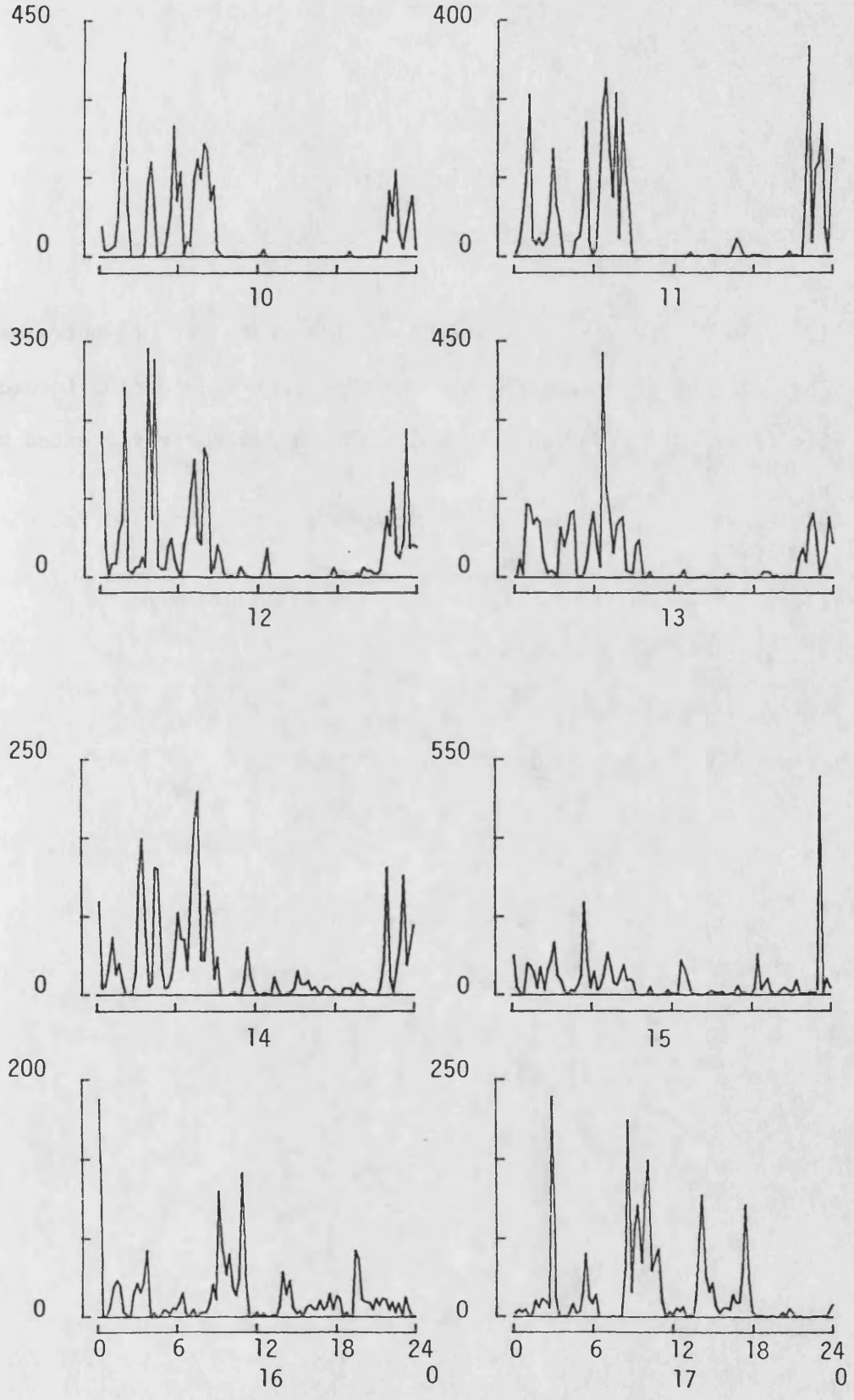

450
0
10
400
0
11
350
0
12
450
0
13
250
0
14
550
0
15
200
0
0 6 12 18 24
16
250
0
0 6 12 18 24
17
0

Fig. 5.7b. Effect of melatonin on the rate of re-entrainment following a phase inversion of the LD cycle: sequence of locomotor activity profiles over 24 h from day 18 to 25 for rat treated with saline.

For actogram, see Fig 5.5b.

Abscissa: Time (h). Ordinate: Counts per epoch (15 min).

Day number depicted beneath abscissa.

Initial LD cycle: 12:12 (lights on: 09:00 h)

Final LD cycle: 12:12 (lights on: 21:00 h)

Injections begun: day 12 at 08:30 h

Phase inversion: day 15 at 09:00 h (24 h of dark)

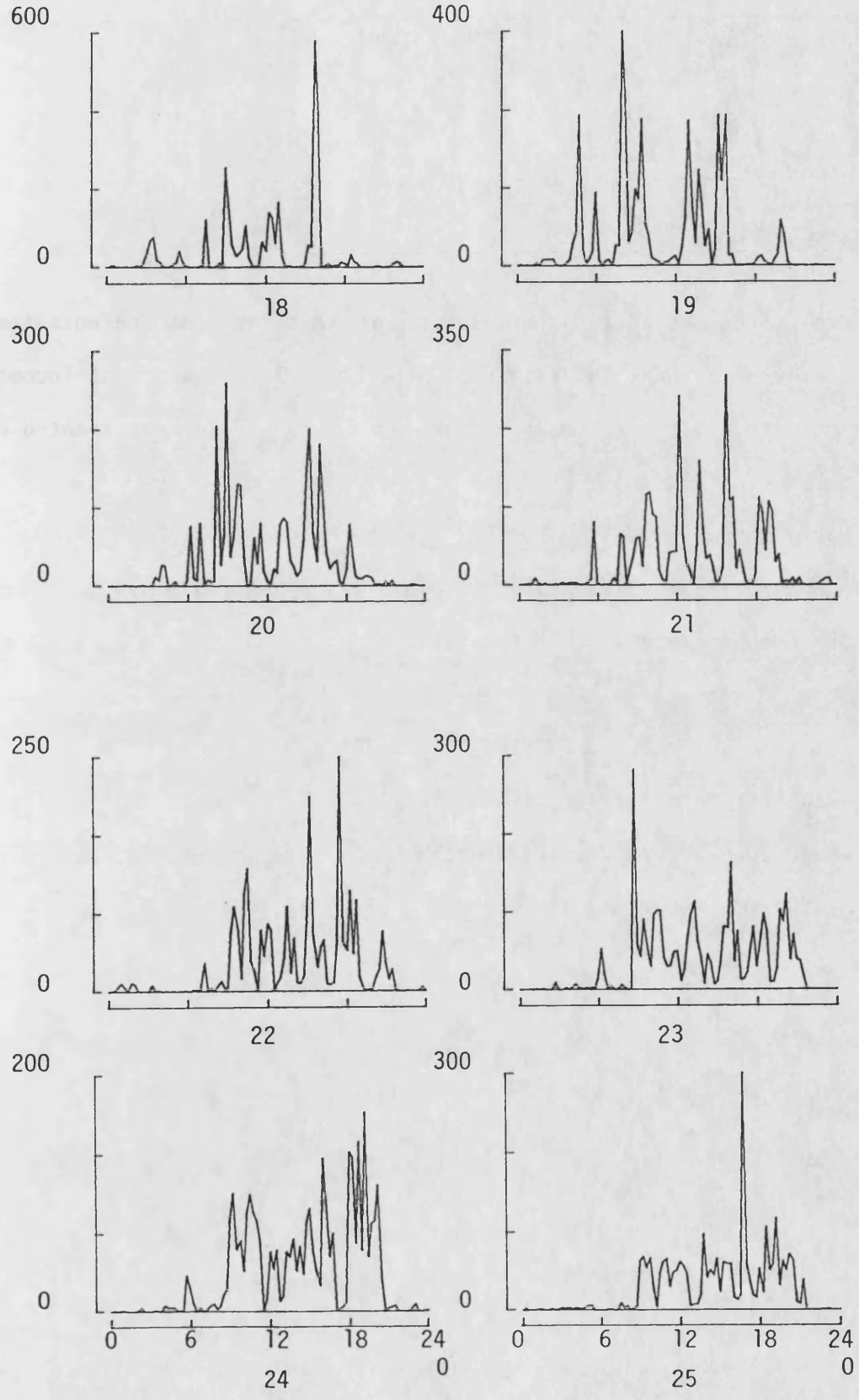

600
0
18
400
0
19
300
0
20
350
0
21
250
0
22
300
0
23
200
0
0
6
12
18
24
0
24
300
0
0
6
12
18
24
0
25

Fig. 5.8a. Effect of melatonin on the rate of re-entrainment following a phase inversion of the LD cycle: sequence of locomotor activity profiles over 24 h from day 10 to 17 for rat treated with melatonin (1mg/kg).

For actogram, see Fig 5.5a.

Abscissa: Time (h). Ordinate: Counts per epoch (15 min).

Day number depicted beneath abscissa.

Initial LD cycle: 12:12 (lights on: 09:00 h)

Final LD cycle: 12:12 (lights on: 21:00 h)

Injections begun: day 12 at 08:30 h

Phase inversion: day 15 at 09:00 h (24 h of dark)

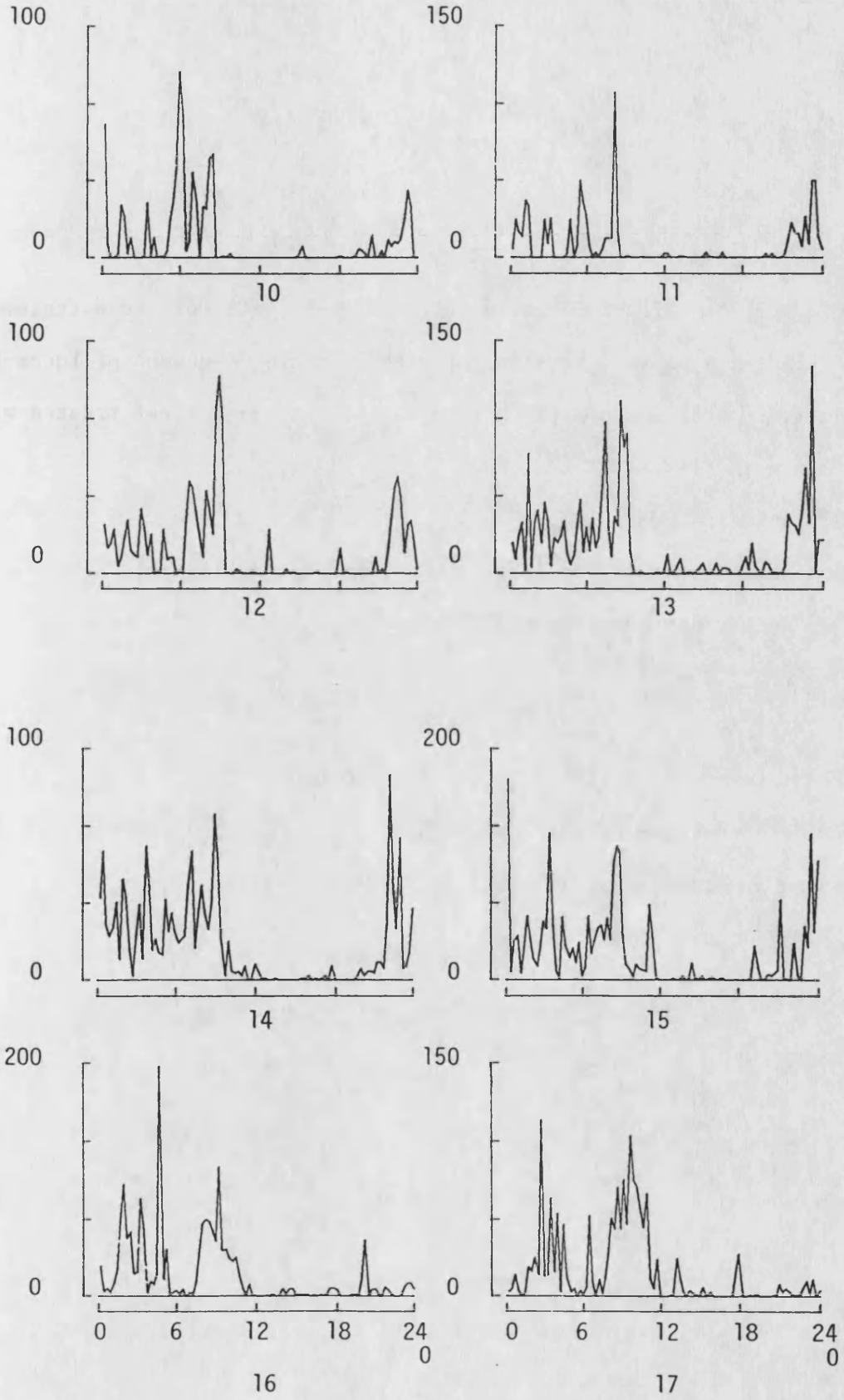

100
0
10
150
0
11
100
0
12
150
0
13
100
0
14
200
0
15
200
0
0
6
12
18
24
0
16
150
0
0
6
12
18
24
0
17

Fig. 5.8b. Effect of melatonin on the rate of re-entrainment following a phase inversion of the LD cycle: sequence of locomotor activity profiles over 24 h from day 18 to 25 for rat treated with melatonin (1mg/kg).

For actogram, see Fig 5.5a.

Abscissa: Time (h). Ordinate: Counts per epoch (15 min).

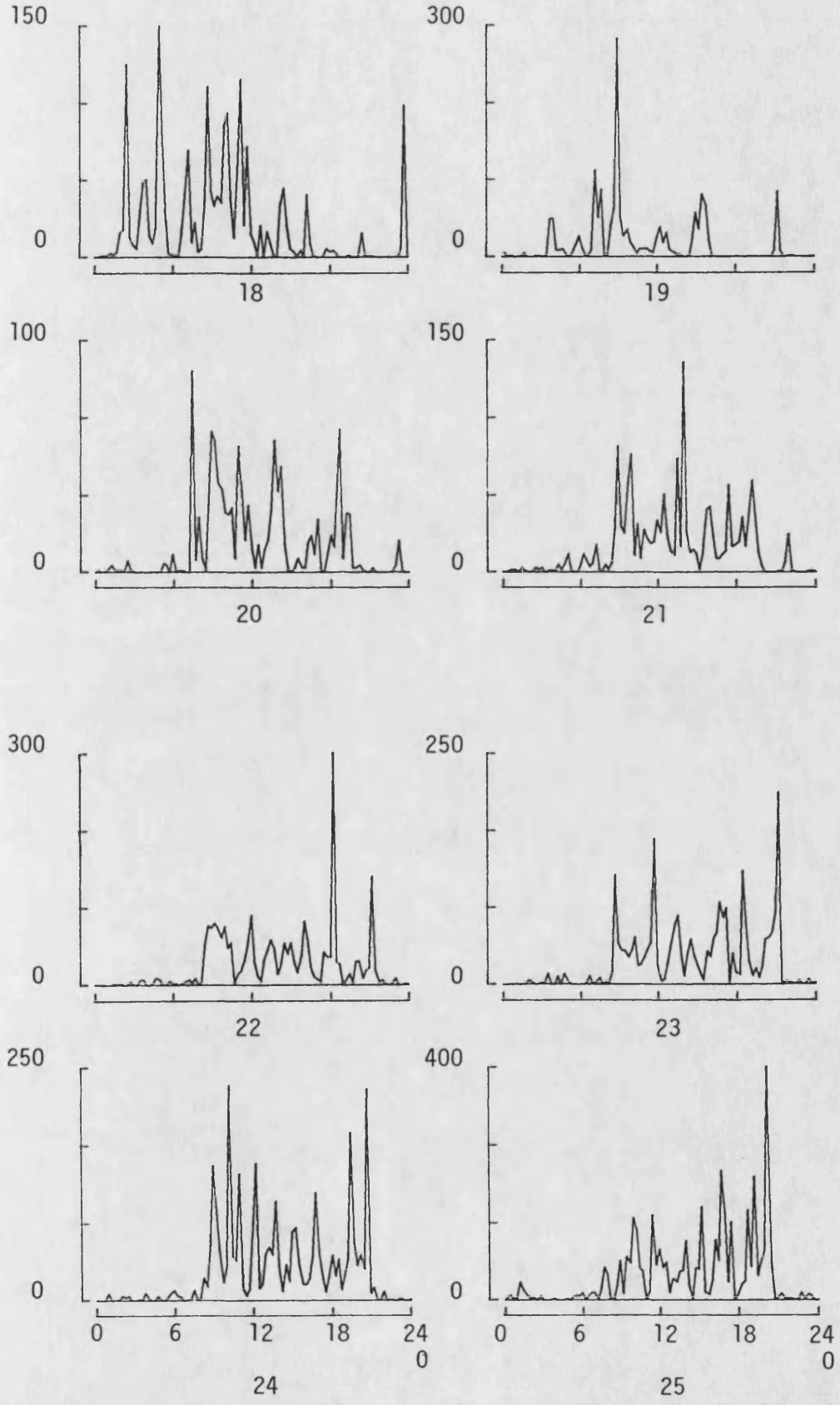
Day number depicted beneath abscissa.

Initial LD cycle: 12:12 (lights on: 09:00 h)

Final LD cycle: 12:12 (lights on: 21:00 h)

Injections begun: day 12 at 08:30 h

Phase inversion: day 15 at 09:00 h (24 h of dark)


150
0
18
300
0
19
100
0
20
150
0
21
300
0
22
250
0
23
250
0
0
6
12
18
24
0
24
400
0
0
6
12
18
24
0
25

5.5.2. Experiment 2: Effect of melatonin on free-running locomotor activity rhythms.

A degree of entrainment was obtained in all animals treated with melatonin. Visual examination of the actogram trace produced by one melatonin-treated animal suggests that entrainment to an approximate 24 h period persisted after cessation of therapy (see Fig. 5.9d), although it is not possible to determine whether this simply reflects a reduced free-running period induced by melatonin since a further trace depicts a similar but less pronounced change (see Fig. 5.9e). One animal of the control group entrained to the treatment - entrainment persisting beyond the last day of injection to the end of the experiment (see Fig. 5.9b). One saline-treated animal displayed partial de-synchronization as depicted in Fig. 5.9j. Periodogram analysis of the data failed to substantiate estimates of the periods made by visual inspection of actograms (see Figs. 5.10a-5.10i and also Figs. 5.11a and 5.11b). Periods, as revealed by periodogram analysis, are shown in Tables 5.1 and 5.2.

Fig. 5.9a. Effect of melatonin on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 1.

Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram. Activity monitored using Animex.

DD from day 1.

Treatment: saline at 03:15 h, days 48-61 inclusive.

ACTOGRAM

BOX 1

THRESHOLD=50% SAMPLING INTERVAL=15Min

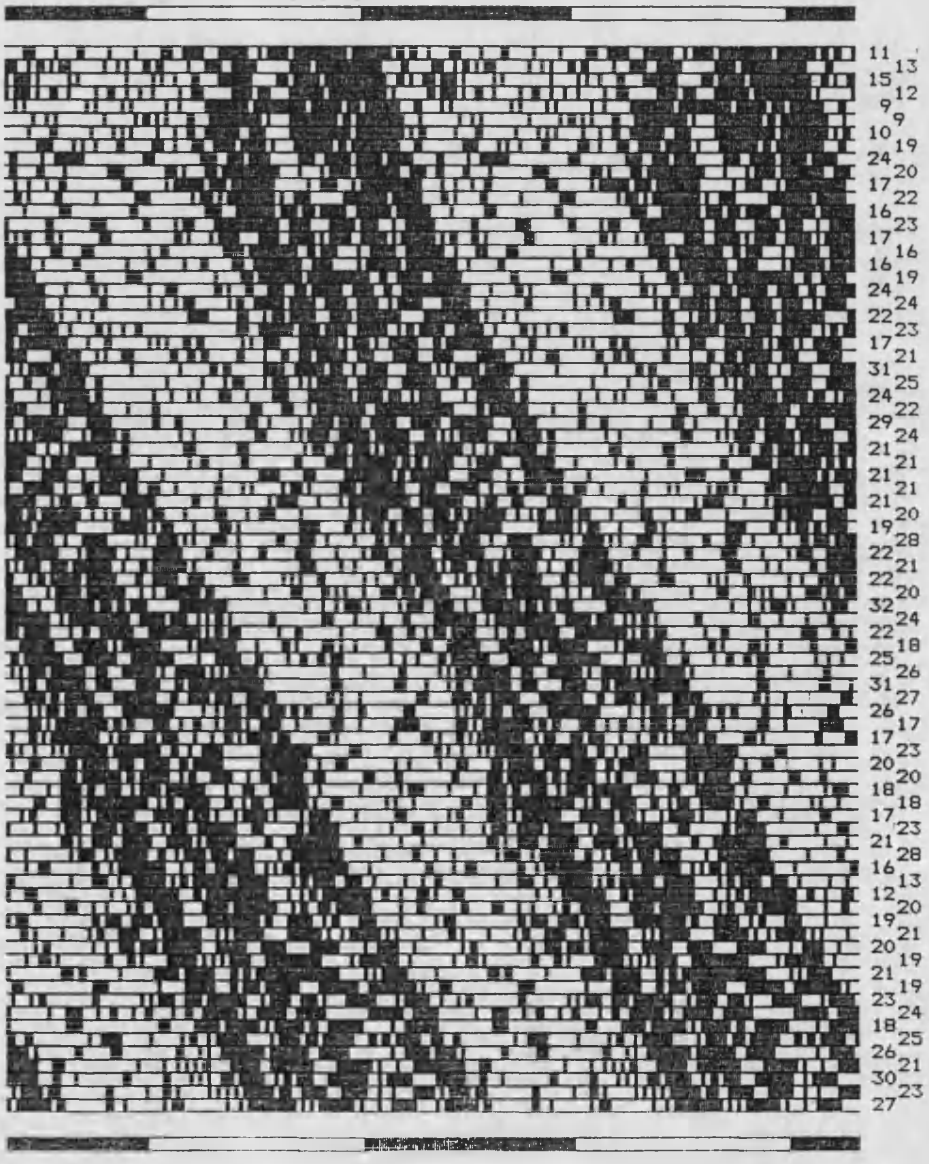


Fig. 5.9b. Effect of melatonin on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 2.

Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram. Activity monitored using ANIMEX.

DD from day 1.

Treatment: saline at 18:00 h, days 47-60 inclusive.

ACTOGRAM

BOX 2

THRESHOLD=50% SAMPLING INTERVAL=15Min

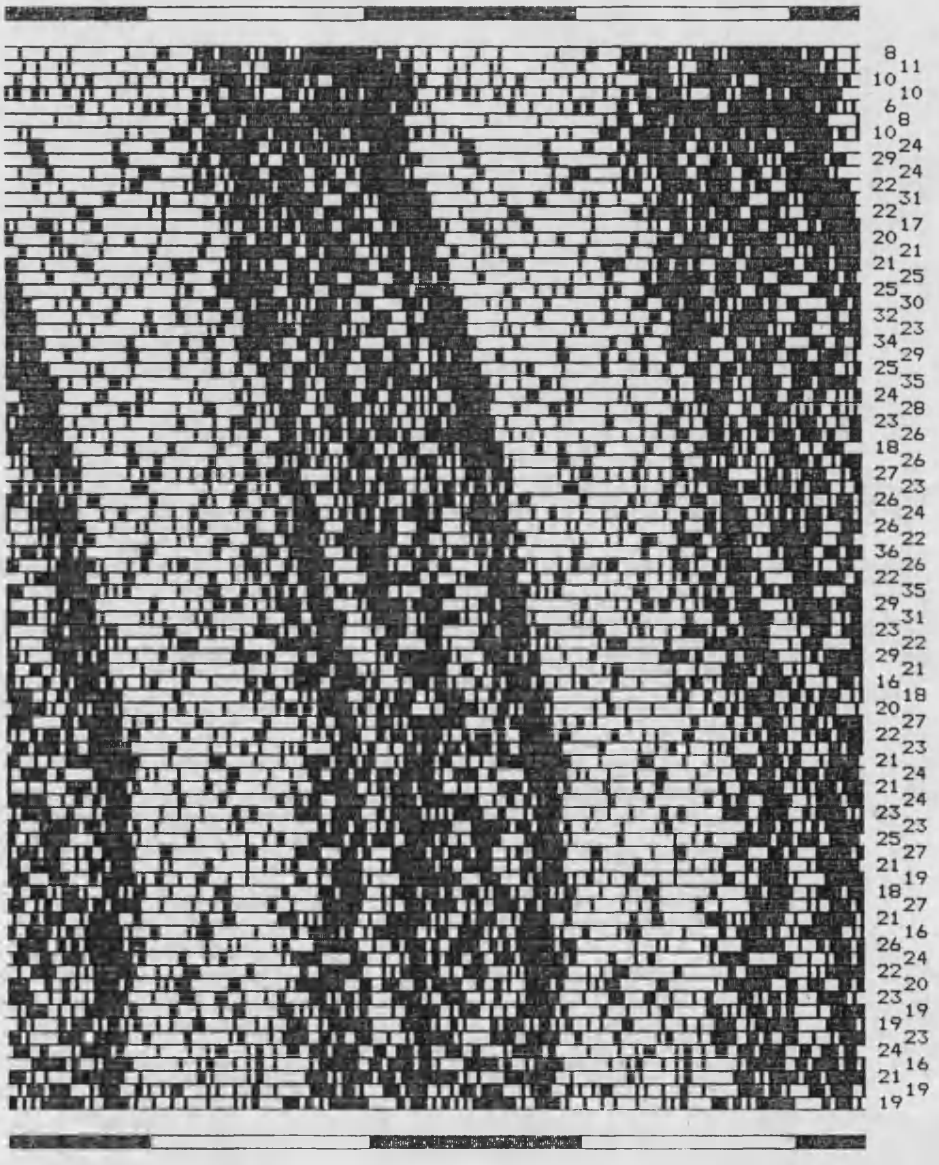


Fig. 5.9c. Effect of melatonin on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 3.

Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram. Activity monitored using ANIMEX.

DD from day 1.

Treatment: melatonin 1mg/kg at 18:00 h, days 47-60 inclusive.

ACTOGRAM

BOX 3

THRESHOLD=50% SAMPLING INTERVAL=15Min

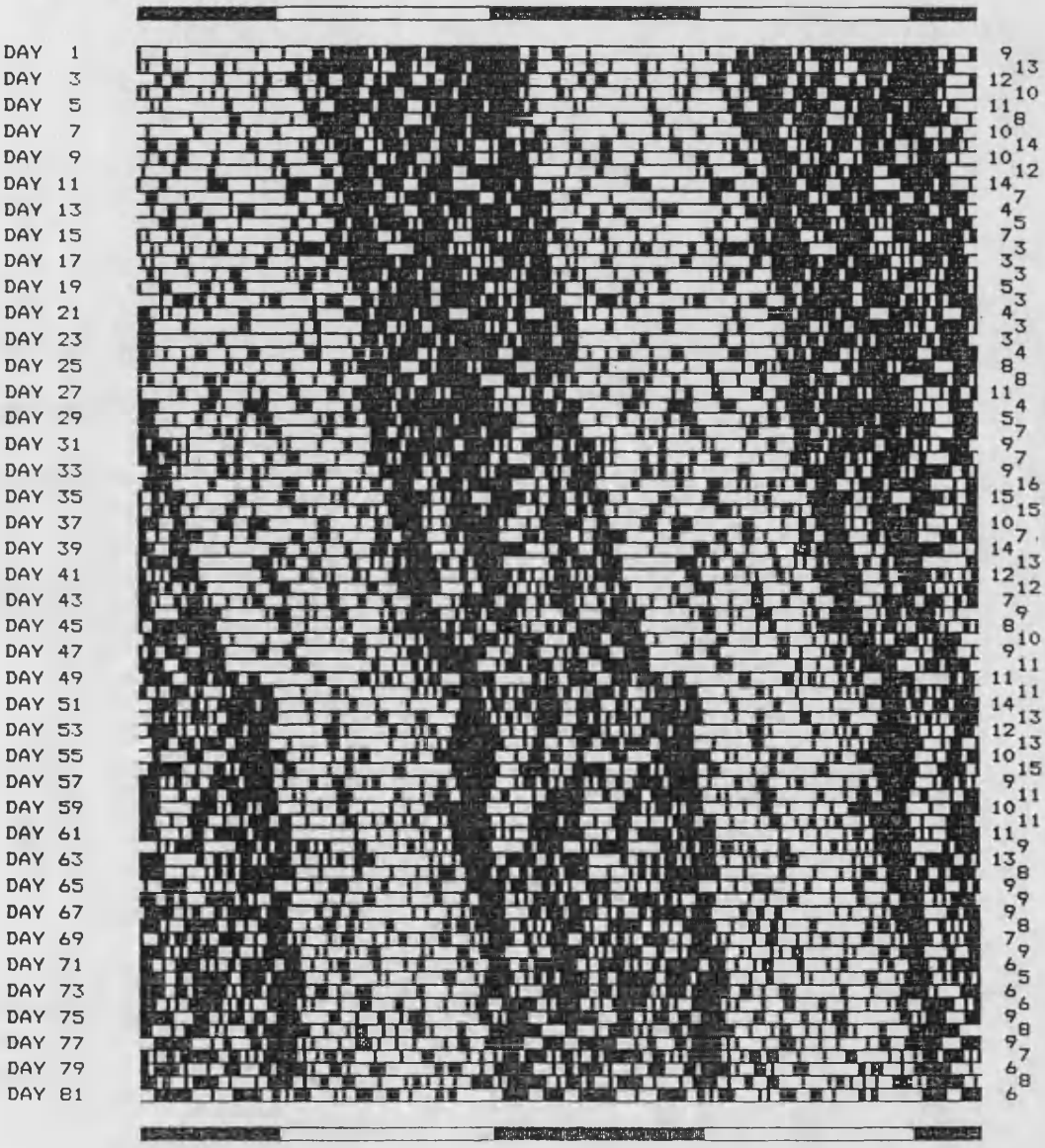


Fig. 5.9d. Effect of melatonin on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 4.

Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram. Activity monitored using ANIMEX.

DD from day 1.

Treatment: melatonin 1mg/kg at 10:15 h, days 47-60 inclusive.

ACTOGRAM

BOX 4

THRESHOLD=50% SAMPLING INTERVAL=15Min

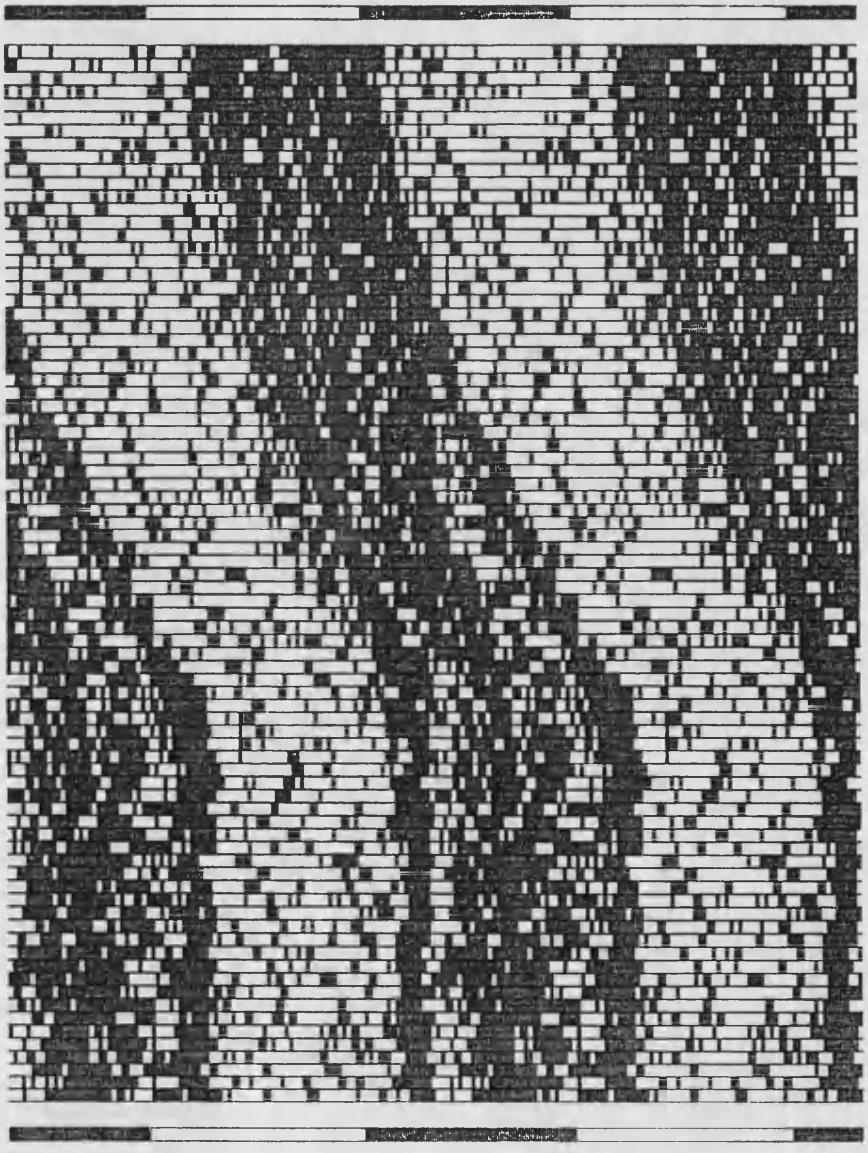


Fig. 5.9e. Effect of melatonin on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 5.

Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram. Activity monitored using PHOTOCELLS.

DD from day 1.

Treatment: melatonin 1mg/kg at midnight, days 47-60 inclusive.

Fig. 5.9f. Effect of melatonin on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 6.

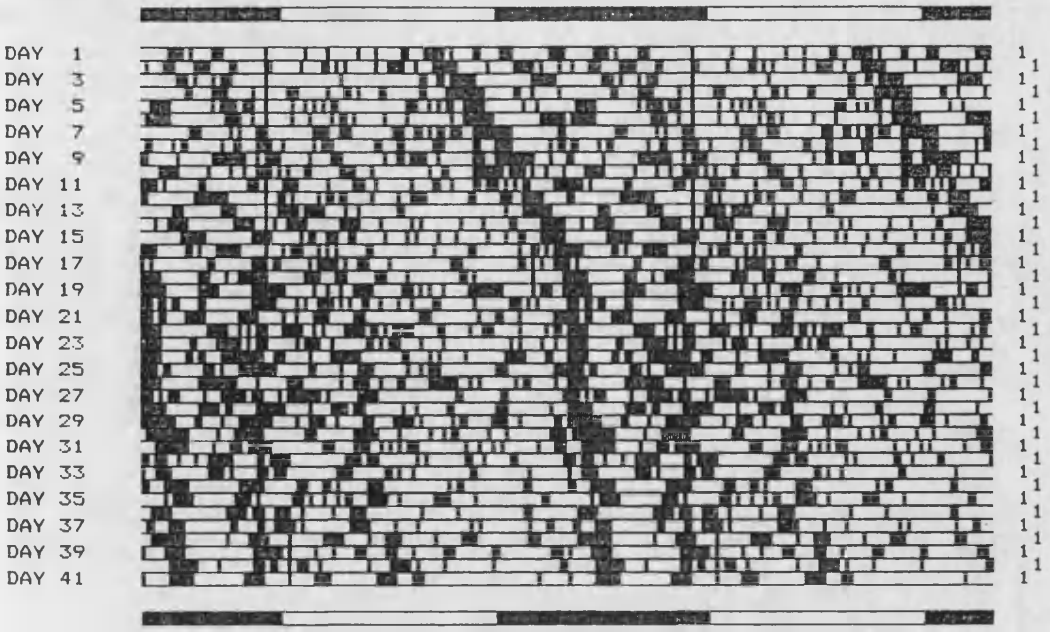
Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram. Activity monitored using PHOTOCELLS.

DD from day 1.

Animal withdrawn at end of day 23, no injections given.

ACTOGRAM

BOX 1
THRESHOLD=50% SAMPLING INTERVAL=15Min



ACTOGRAM

BOX 2
THRESHOLD=50% SAMPLING INTERVAL=15Min

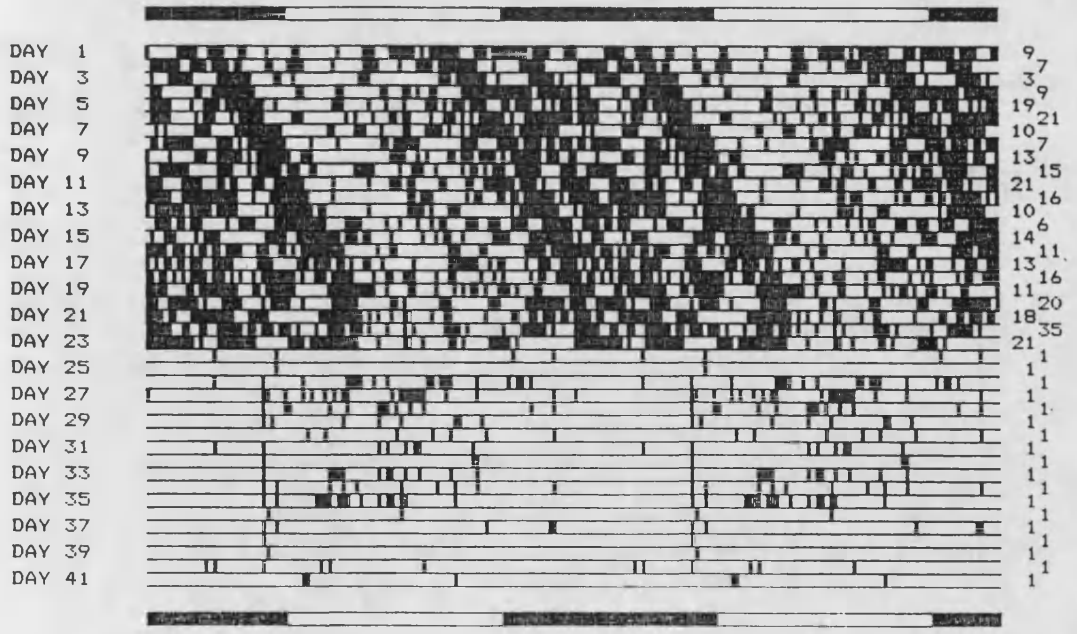


Fig. 5.9g. Effect of melatonin on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 7.

Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram. Activity monitored using PHOTOCELLS.

DD from day 1.

Treatment: melatonin 1mg/kg at 21:30 h, days 14-27 inclusive.

Fig. 5.9h. Effect of melatonin on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 8.

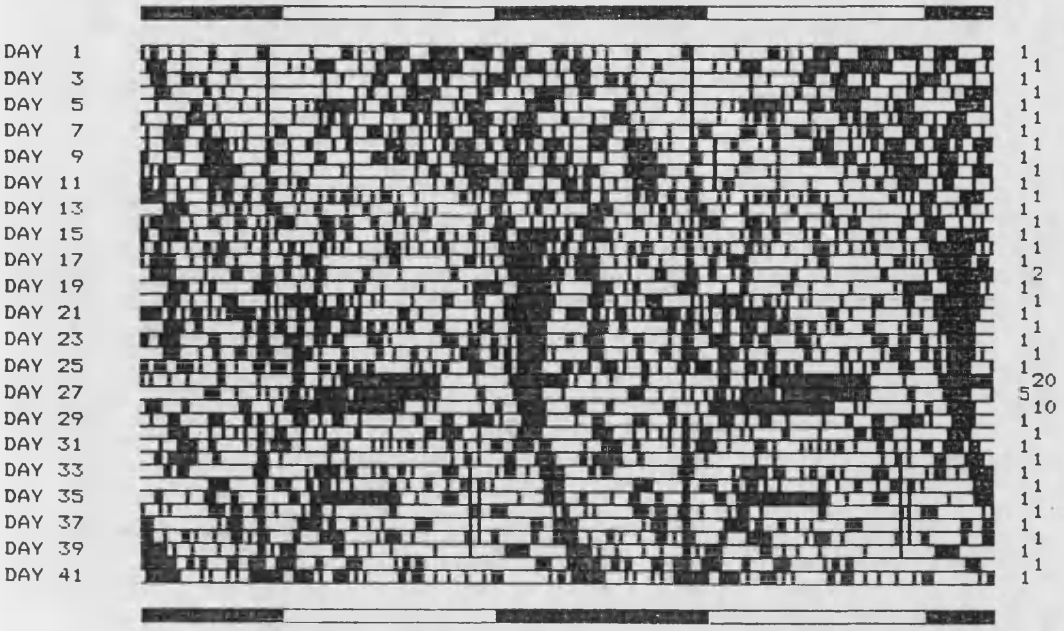
Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram. Activity monitored using PHOTOCELLS.

DD from day 1

Treatment: saline at 10:00 h, days 14-27 inclusive.

ACTOGRAM

BOX 3
THRESHOLD=50% SAMPLING INTERVAL=15Min



ACTOGRAM

BOX 4
THRESHOLD=50% SAMPLING INTERVAL=15Min

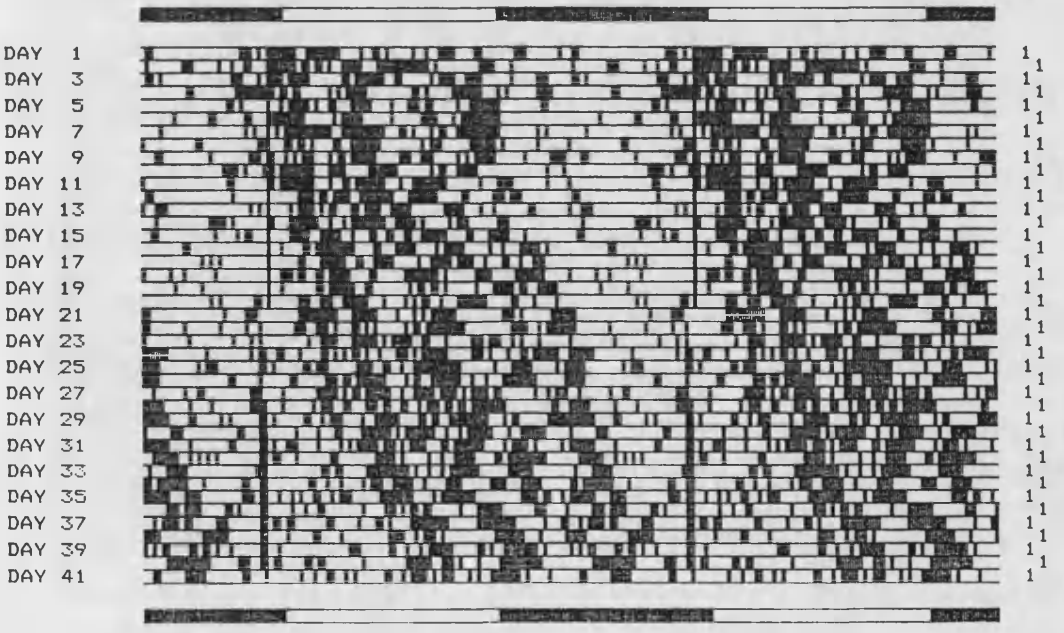


Fig. 5.9i. Effect of melatonin on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 9.

Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram. Activity monitored using PHOTOCELLS.

DD from day 1

Treatment: melatonin 1mg/kg at 17:00 h, days 14-27 inclusive.

Fig. 5.9j. Effect of melatonin on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 10.

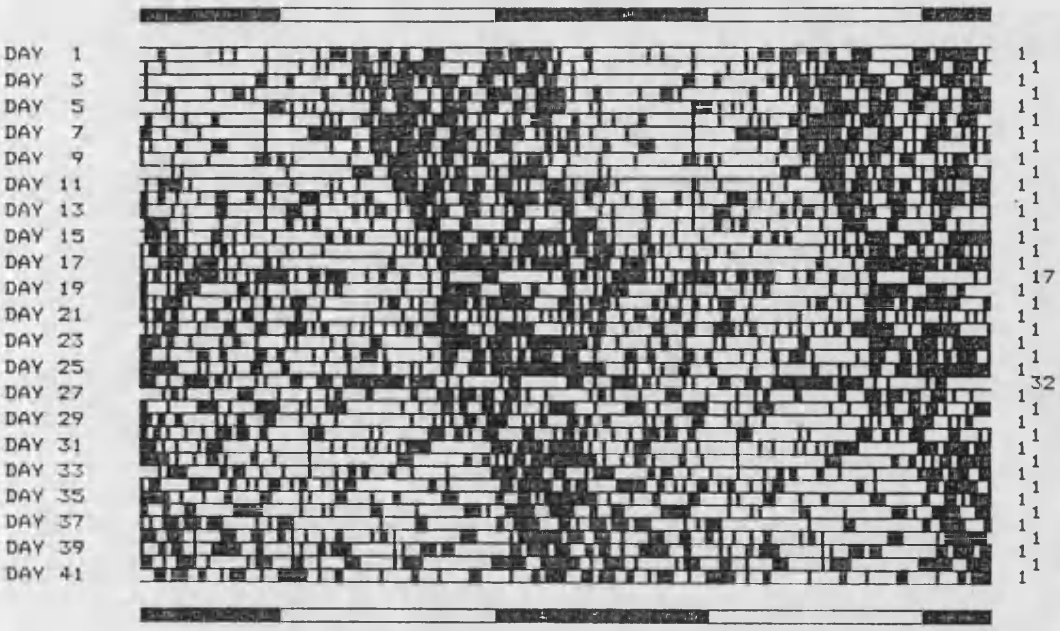
Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram. Activity monitored using PHOTOCELLS.

DD from day 1

Treatment: saline at 20:30 h, days 14-27 inclusive.

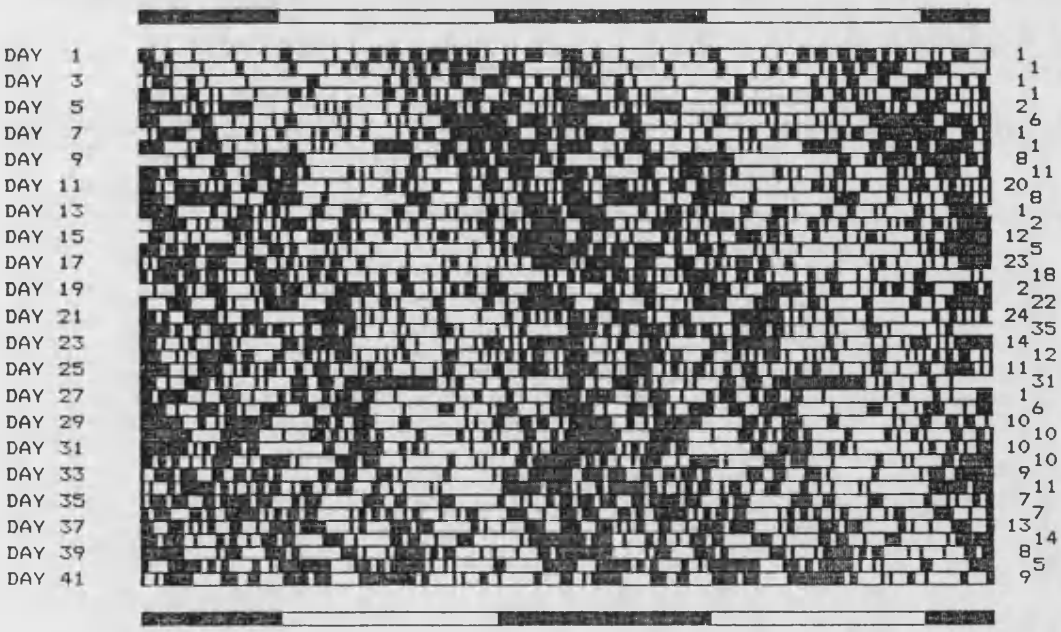
ACTOGRAM

BOX 5
THRESHOLD=50% SAMPLING INTERVAL=15Min



ACTOGRAM

BOX 6
THRESHOLD=50% SAMPLING INTERVAL=15Min



Animal	T	Interval Analysed (days)	Predominant Periods (h) Secondaries	Fundamental	
1	Pre	10 (37-46)	3.50, 6.00	24.25	(24.35)
	S	10 (50-59)	12.00	24.50	(24.35)
	Post	10 (62-71)	3.50, 28.50	24.75	(24.35)
	Pre	20 (25-44)	22.75	24.50	
	Post	20 (62-81)	3.25, 12.25	24.50	
2	Pre	10 (37-46)	4.00, 6.00, 12.00	23.75	(24.15)
	S	10 (50-59)	4.00, 6.00, 12.25	23.75	(24.00)
	Post	10 (62-71)	3.50, 4.00, 6.00	23.75	(24.00)
	Pre	20 (25-44)	6.00, 12.00	24.00	
	Post	20 (62-81)	4.00, 6.00, 12.00	23.75	
3	Pre	10 (37-46)	4.00, 6.00, 12.00	23.75	(24.20)
	M	10 (50-59)	4.00, 6.00, 12.00	23.50	(24.00)
	Post	10 (62-71)	4.00, 6.00, 12.00	23.75	(24.10)
	Pre	20 (25-44)	6.00, 12.00	24.25	
	Post	20 (62-81)	4.00, 4.75, 6.00, 12.00	24.00	
4	Pre	10 (37-46)	3.50, 5.00, 21.00	24.00	(24.30)
	M	10 (50-59)	4.00, 6.00, 12.00	23.75	(24.00)
	Post	10 (62-71)	3.00, 6.00, 12.00	24.00	(24.00)
	Pre	20 (25-44)	22.75	24.25	
	Post	20 (62-81)	3.00, 4.00, 6.00	24.00	

Table 5.1. Effect of melatonin on free-running circadian rhythms: periodogram analysis of the circadian rhythm of locomotor activity expressed by rats using the ANIMEX activity monitoring system.

T=Treatment, Pre=Pre-treatment period, S=Saline, M=melatonin 1mg/kg, Post=Post-treatment period.

Interval analysis: DD from day 1; values in parenthesis indicate days used for spectral analysis.

Periodogram analysis: values indicate the fundamental and secondary periods of rhythmic activity. Visual estimate of periods are shown in parenthesis.

The actogram and derived periodogram functions are shown in Figs. 5.9a-5.9d and 5.10a-5.10d respectively.

Animal	T	Interval Analysed (days)	Predominant Periods (h) Secondaries	Fundamental
5	Pre	10 (4-13)	3.50, 10.50, 12.25	24.00 (24.40)
	M	10 (17-26)	3.00, 4.00, 6.00	23.75 (24.00)
	Post	10 (29-38)	4.00, 4.50, 6.00	24.25 (24.10)
6	Animal withdrawn			
7	Pre	10 (4-13)	3.50, 4.50, 12.25	24.50 (24.30)
	M	10 (17-26)	4.00, 6.00, 12.00	23.75 (24.00)
	Post	10 (29-38)	3.50, 4.75, 12.00	24.25 (24.30)
8	Pre	10 (4-13)	4.00, 4.50, 28.00	24.00 (24.20)
	S	10 (17-26)	6.00	24.25 (24.20)
	Post	10 (29-38)	4.00, 4.75, 12.25	24.25 (24.20)
9	Pre	10 (4-13)	1.75, 8.00, 21.00	24.00 (24.35)
	M	10 (17-26)	12.50, 15.25, 27.50	23.25 (24.00)
	Post	10 (29-38)	4.25, 12.00	24.00 (24.20)
10	Pre	10 (4-13)	12.50, 21.25	24.25 (N/E)
	S	10 (17-26)	13.50	23.75 (N/E)
	Post	10 (29-38)	6.00, 8.00, 12.00	23.75 (N/E)

Table 5.2. Effect of melatonin on free-running circadian rhythms: periodogram analysis of the circadian rhythm of locomotor activity expressed by rats using the PHOTOCELL activity monitoring system.

T=Treatment, Pre=Pre-treatment period, S=Saline, M=melatonin 1mg/kg, Post=Post-treatment period.

Interval analysis: DD from day 1; values in parenthesis indicate days used for spectral analysis.

Periodogram analysis: values indicate the fundamental and secondary periods of rhythmic activity. Visual estimate of periods are shown in parenthesis (N/E=Not Estimated).

The actogram and derived periodogram functions are shown in Figs. 5.9e-5.9j and 5.10e-5.10i respectively.

Fig. 5.10a. Effect of melatonin on free-running circadian rhythms: periodogram analysis of rat 1.

For actogram, see Fig. 5.9a.

Abscissa: Period length (h). Ordinate: Relative power ($x10^3$).

Treatment: saline at 03:15 h, days 48-61.

a). DD days 37-46 (pre-treatment).

b). DD days 50-59 (during treatment).

c). DD days 62-71 (post-treatment).

Fig. 5.10b. Effect of melatonin on free-running circadian rhythms: periodogram analysis of rat 2.

For actogram, see Fig. 5.9b.

Abscissa: Period length (h). Ordinate: Relative power ($x10^3$).

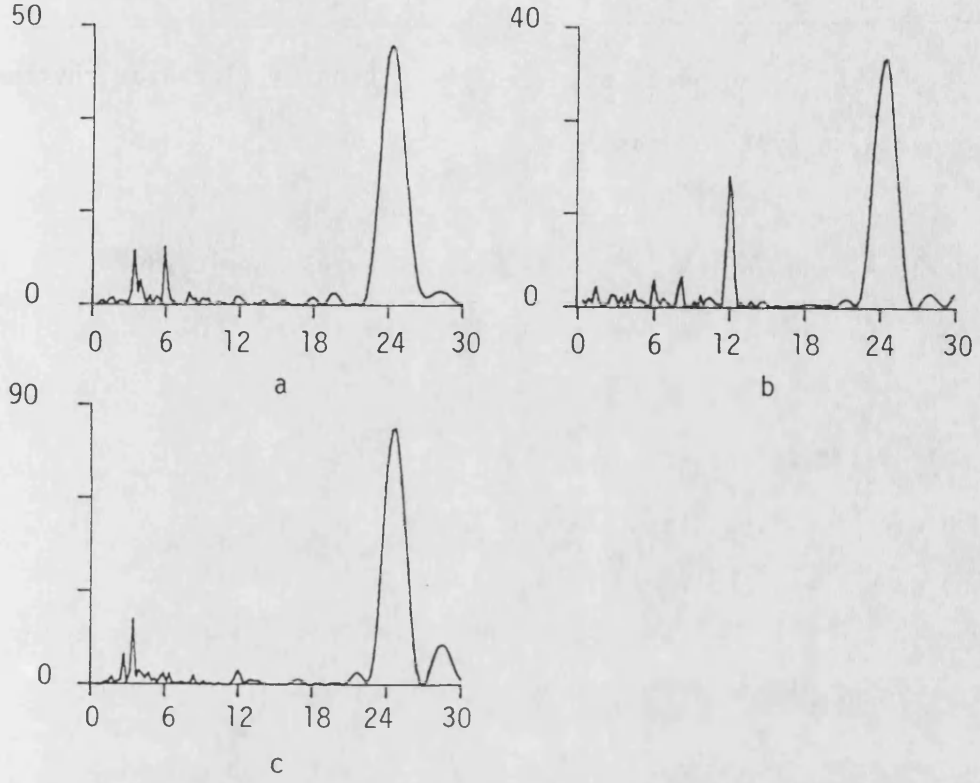
Treatment: saline at 18:00 h, days 47-60.

a). DD days 37-46 (pre-treatment).

b). DD days 50-59 (during treatment).

c). DD days 62-71 (post-treatment).

For a summary of the derived periodogram function, see Table 5.2.


50
0
0 6 12 18 24 30
a
40
0
0 6 12 18 24 30
b
90
0
0 6 12 18 24 30
c

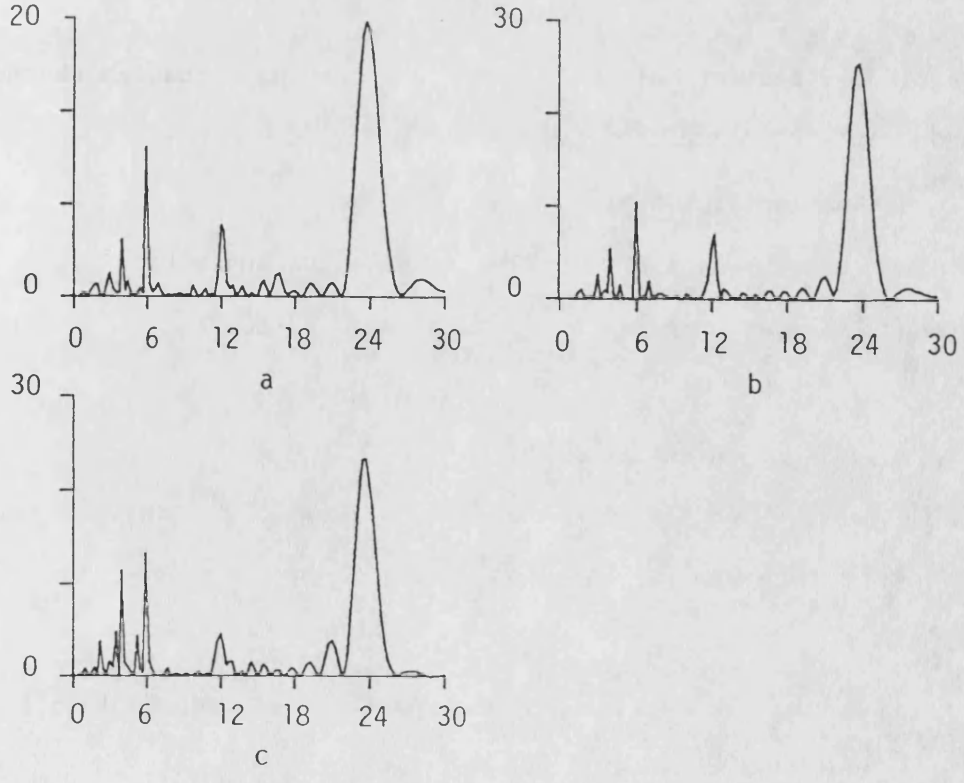

20
0
0 6 12 18 24 30
a
30
0
0 6 12 18 24 30
b
30
0
0 6 12 18 24 30
c

Fig. 5.10c. Effect of melatonin on free-running circadian rhythms: periodogram analysis of rat 3.

For actogram, see Fig. 5.9c.

Abscissa: Period length (h). Ordinate: Relative power ($x10^3$).

Treatment: melatonin 1mg/kg at 18:00 h, days 47-60.

a). DD days 37-46 (pre-treatment).

b). DD days 50-59 (during treatment).

c). DD days 62-71 (post-treatment).

Fig. 5.10d. Effect of melatonin on free-running circadian rhythms: periodogram analysis of rat 4.

For actogram, see Fig. 5.9d.

Abscissa: Period length (h). Ordinate: Relative power ($x10^3$).

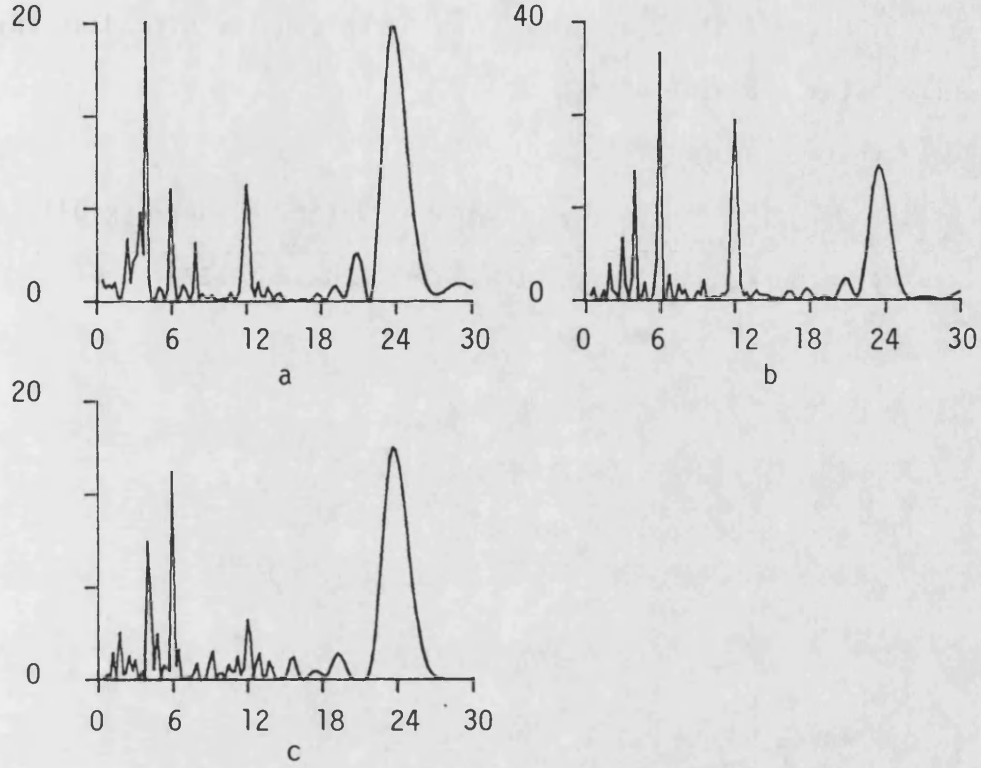
Treatment: melatonin 1mg/kg at 10:15 h, days 47-60.

a). DD days 37-46 (pre-treatment).

b). DD days 50-59 (during treatment).

c). DD days 62-71 (post-treatment).

For a summary of the derived periodogram function, see Table 5.2.


20
0
0 6 12 18 24 30
a
40
0
0 6 12 18 24 30
b
20
0
0 6 12 18 24 30
c

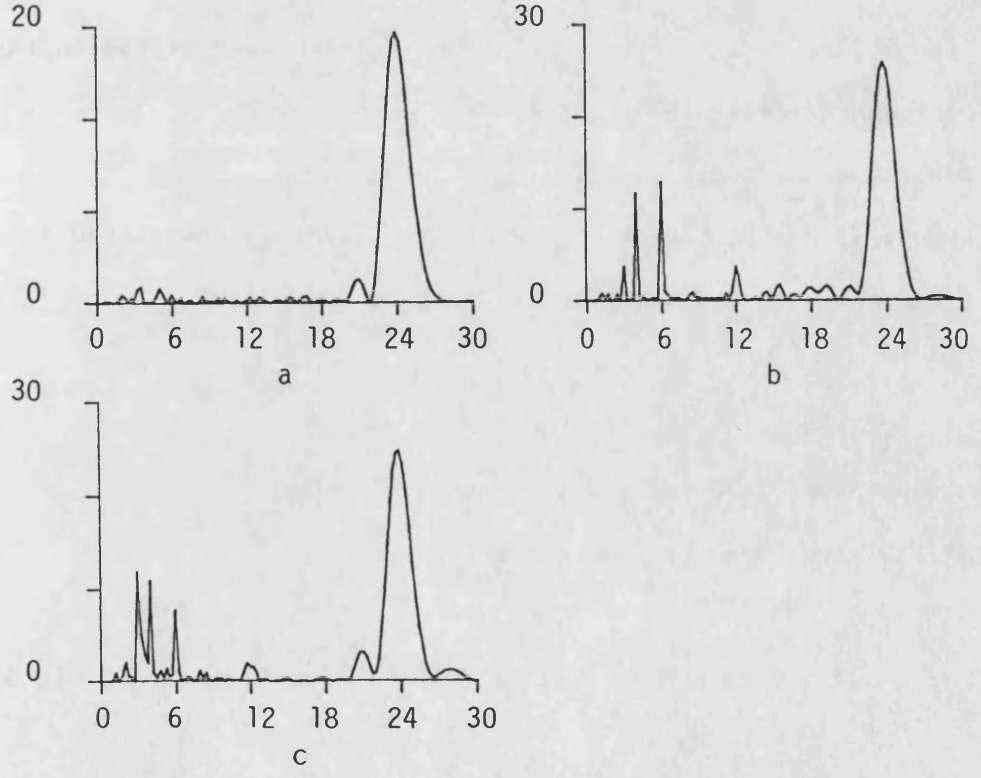

20
0
0 6 12 18 24 30
a
30
0
0 6 12 18 24 30
b
30
0
0 6 12 18 24 30
c

Fig. 5.10e. Effect of melatonin on free-running circadian rhythms: periodogram analysis of rat 5.

For actogram, see Fig. 5.9e.

Abscissa: Period length (h). Ordinate: Relative power ($x10^3$).

Treatment: melatonin 1mg/kg at midnight, days 14-27.

a). DD days 4-13 (pre-treatment).

b). DD days 17-26 (during treatment).

c). DD days 29-38 (post-treatment).

Fig. 5.10f. Effect of melatonin on free-running circadian rhythms: periodogram analysis of rat 7.

For actogram, see Fig. 5.9g.

Abscissa: Period length (h). Ordinate: Relative power ($x10^3$).

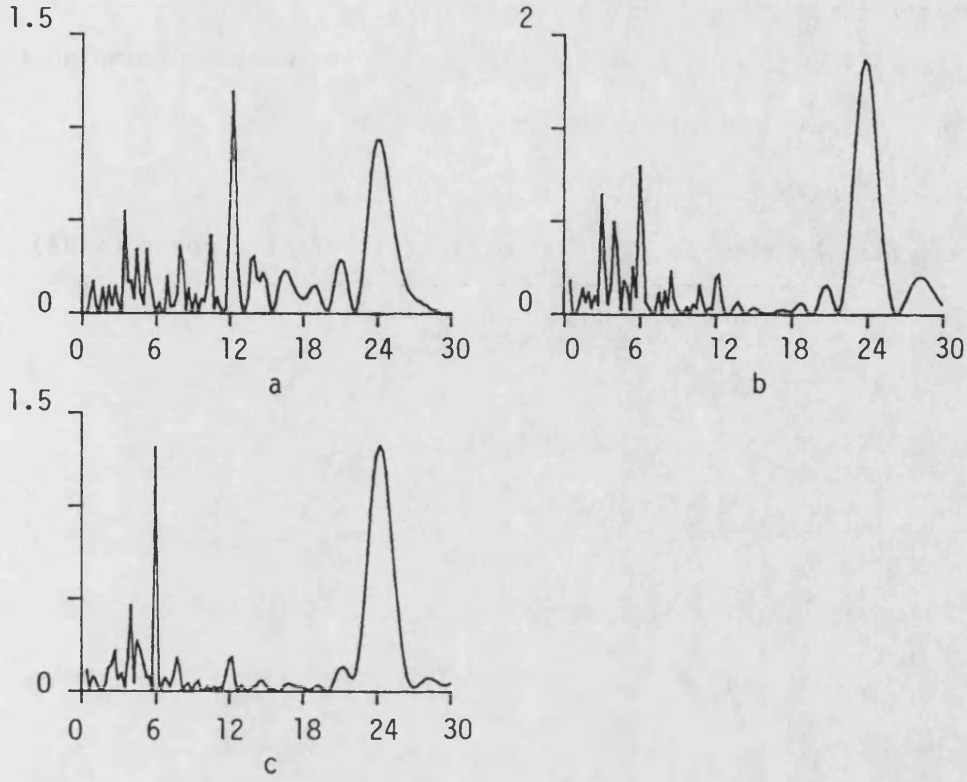
Treatment: melatonin 1mg/kg at 21:30 h, days 14-27.

a). DD days 4-13 (pre-treatment).

b). DD days 17-26 (during treatment).

c). DD days 29-38 (post-treatment).

For a summary of the derived periodogram function, see Table 5.2.


1.5
0
0 6 12 18 24 30
a
2
0
0 6 12 18 24 30
b
1.5
0
0 6 12 18 24 30
c

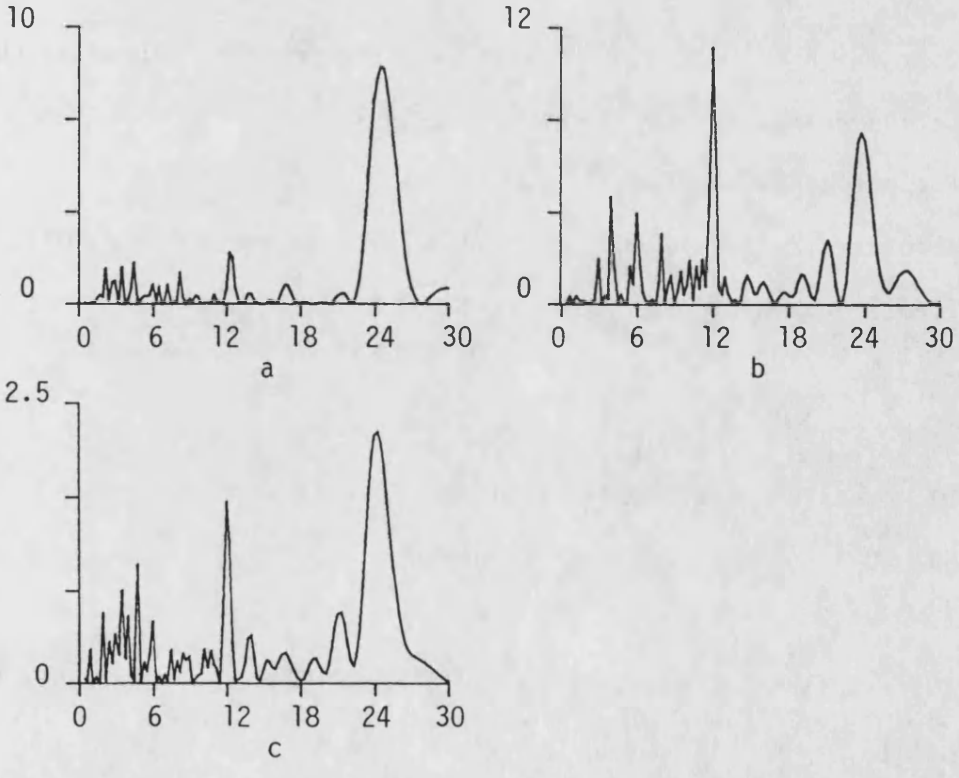

10
0
0 6 12 18 24 30
a
12
0
0 6 12 18 24 30
b
2.5
0
0 6 12 18 24 30
c

Fig. 5.10g. Effect of melatonin on free-running circadian rhythms: periodogram analysis of rat 8.

For actogram, see Fig. 5.9h.

Abscissa: Period length (h). Ordinate: Relative power ($x10^3$).

Treatment: saline at 10:00 h, days 14-27.

a). DD days 4-13 (pre-treatment).

b). DD days 17-26 (during treatment).

c). DD days 29-38 (post-treatment).

Fig. 5.10h. Effect of melatonin on free-running circadian rhythms: periodogram analysis of rat 9.

For actogram, see Fig. 5.9i.

Abscissa: Period length (h). Ordinate: Relative power ($x10^3$).

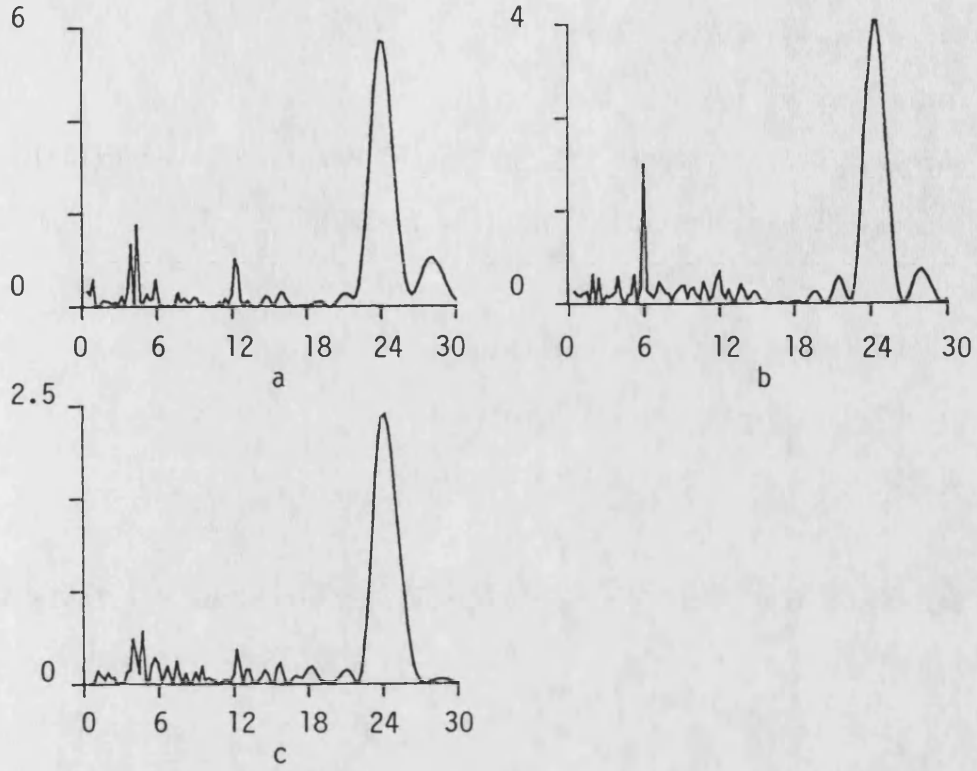
Treatment: melatonin 1mg/kg at 17:00 h, days 14-27.

a). DD days 4-13 (pre-treatment).

b). DD days 17-26 (during treatment).

c). DD days 29-38 (post-treatment).

For a summary of the derived periodogram function, see Table 5.2.


6
0
0 6 12 18 24 30
a
4
0
0 6 12 18 24 30
b
2.5
0
0 6 12 18 24 30
c

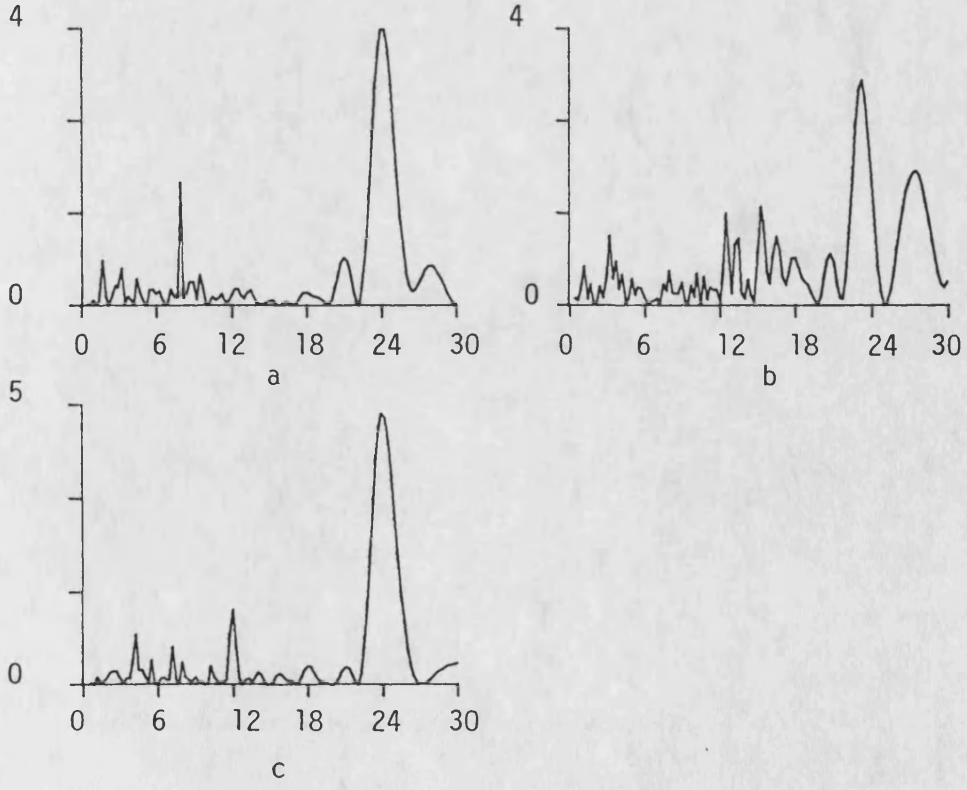

4
0
0 6 12 18 24 30
a
4
0
0 6 12 18 24 30
b
5
0
0 6 12 18 24 30
c

Fig. 5.10i. Effect of melatonin on free-running circadian rhythms: periodogram analysis of rat 10.

For actogram, see Fig. 5.9j.

Abscissa: Period length (h). Ordinate: Relative power ($x10^3$).

Treatment: saline at 20:30 h, days 14-27.

a). DD days 4-13 (pre-treatment).

b). DD days 17-26 (during treatment).

c). DD days 29-38 (post-treatment).

For a summary of the derived periodogram function, see Table 5.2.

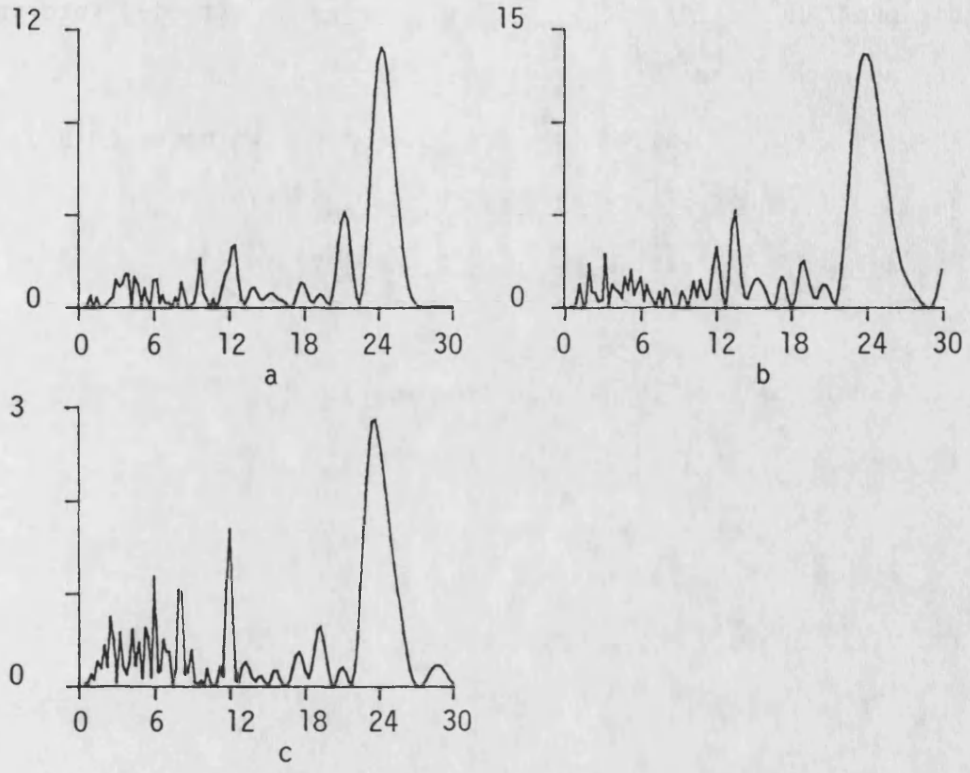

12
0
0 6 12 18 24 30
a
15
0
0 6 12 18 24 30
b
3
0
0 6 12 18 24 30
c

Fig. 5.11a. Effect of melatonin on free-running circadian rhythms: periodogram analysis of rats 1 and 2 using an extended interval.

For actograms, see Figs. 5.9a and 5.9b.

Abscissa: Period length (h). Ordinate: Relative power ($x10^3$).

Rat 1 Treatment: saline at 03:15 h, days 48-61.

Rat 2 Treatment: saline at 18:00 h, days 47-60.

a). Rat 1, DD days 25-44 (pre-treatment).

b). Rat 1, DD days 62-81 (post-treatment).

c). Rat 2, DD days 25-44 (pre-treatment).

d). Rat 2, DD days 62-81 (post-treatment).

Fig. 5.11b. Effect of melatonin on free-running circadian rhythms: periodogram analysis of rats 3 and 4 using an extended interval.

For actograms, see Figs. 5.9c and 5.9d.

Abscissa: Period length (h). Ordinate: Relative power ($x10^3$).

Rat 3 Treatment: melatonin 1mg/kg at 18:00 h, days 48-61.

Rat 4 Treatment: melatonin 1mg/kg at 10:15 h, days 47-60.

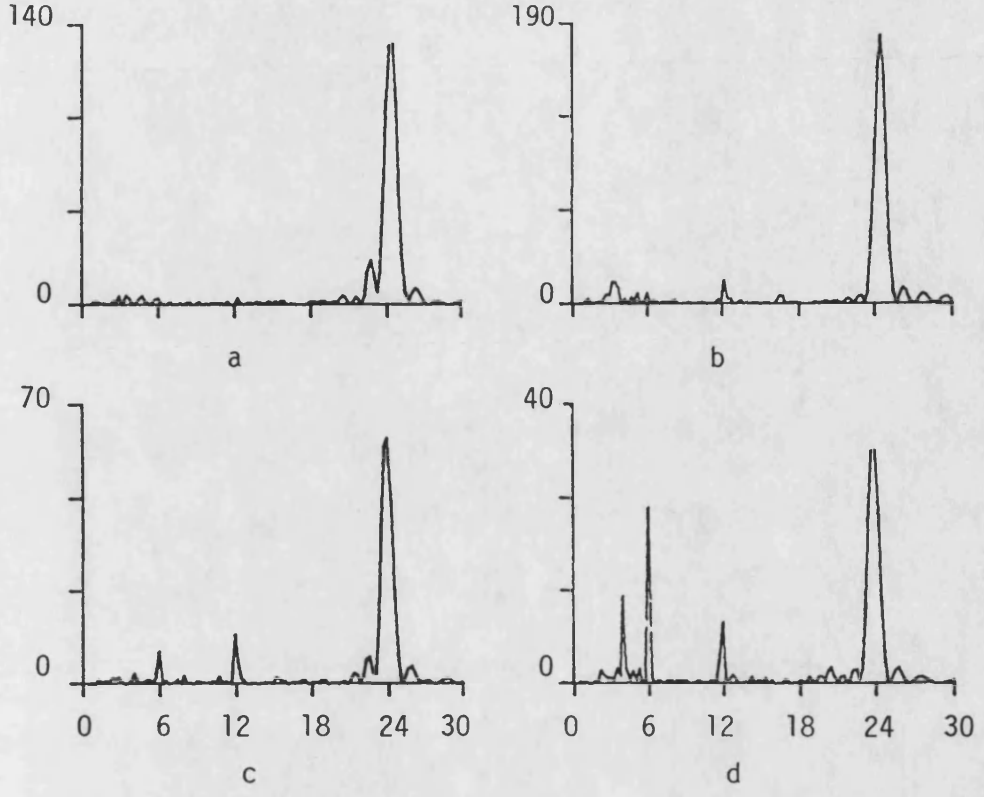
a). Rat 3, DD days 25-44 (pre-treatment).

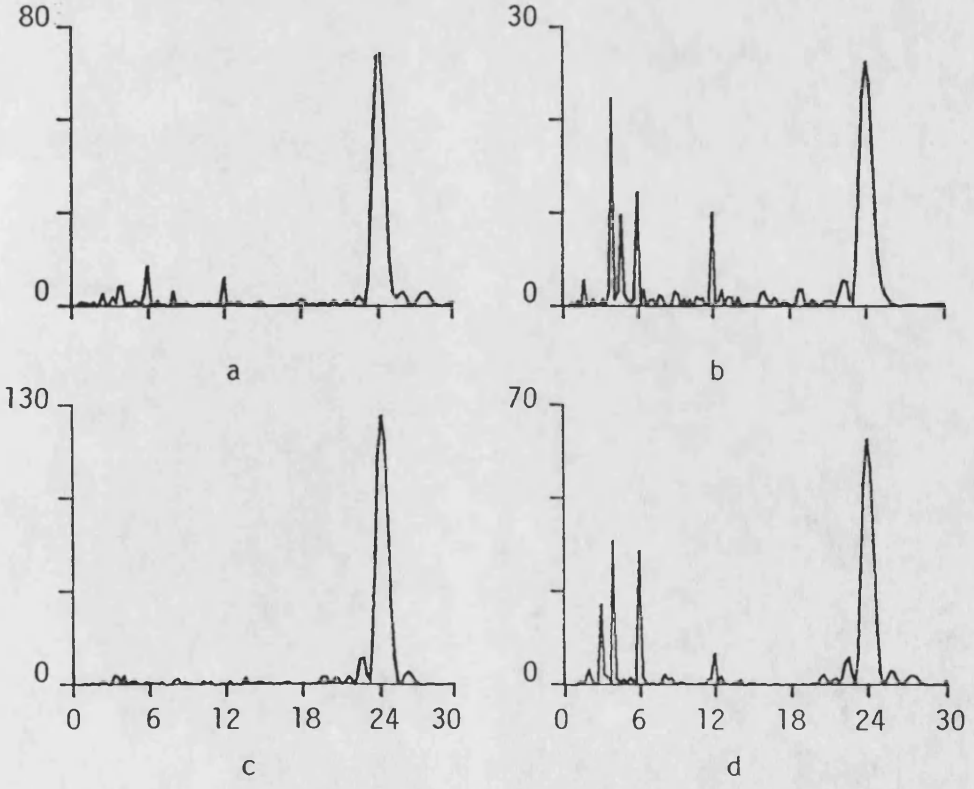
b). Rat 3, DD days 62-81 (post-treatment).

c). Rat 4, DD days 25-44 (pre-treatment).

d). Rat 4, DD days 62-81 (post-treatment).

For a summary of the derived periodogram function, see Table 5.2.


140
0
a
190
0
b
70
0
0 6 12 18 24 30
c
40
0
0 6 12 18 24 30
d


80
0
a
30
0
b
130
0
0 6 12 18 24 30
c
70
0
0 6 12 18 24 30
d

5.5.3. Experiment 3: Effect of melatonin on the phase-shifting ability of light.

Actograms relating to this experiment are shown in Figs. 5.12a-5.12h. A light pulse of 15 min given at CT12 on day 28 failed to effect the anticipated phase-delay of the free-running locomotor activity rhythm in 7 out of 8 animals. Four animals responded to the pulse with an apparent shortening of the free-running period. A phase-delay was obtained in one animal but sickness forced its withdrawal from the experiment (Fig. 5.12b). No further results were obtained in this series of experiments due to equipment failure.

Fig. 5.12a. Effect of melatonin on the phase-shifting ability of light on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 1.

Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram.

DD from day 1

Treatment: 15 min light pulse at 14:45 h (CT12) given on day 28

ACTOGRAM

BOX 1
THRESHOLD=50% SAMPLING INTERVAL=15Min

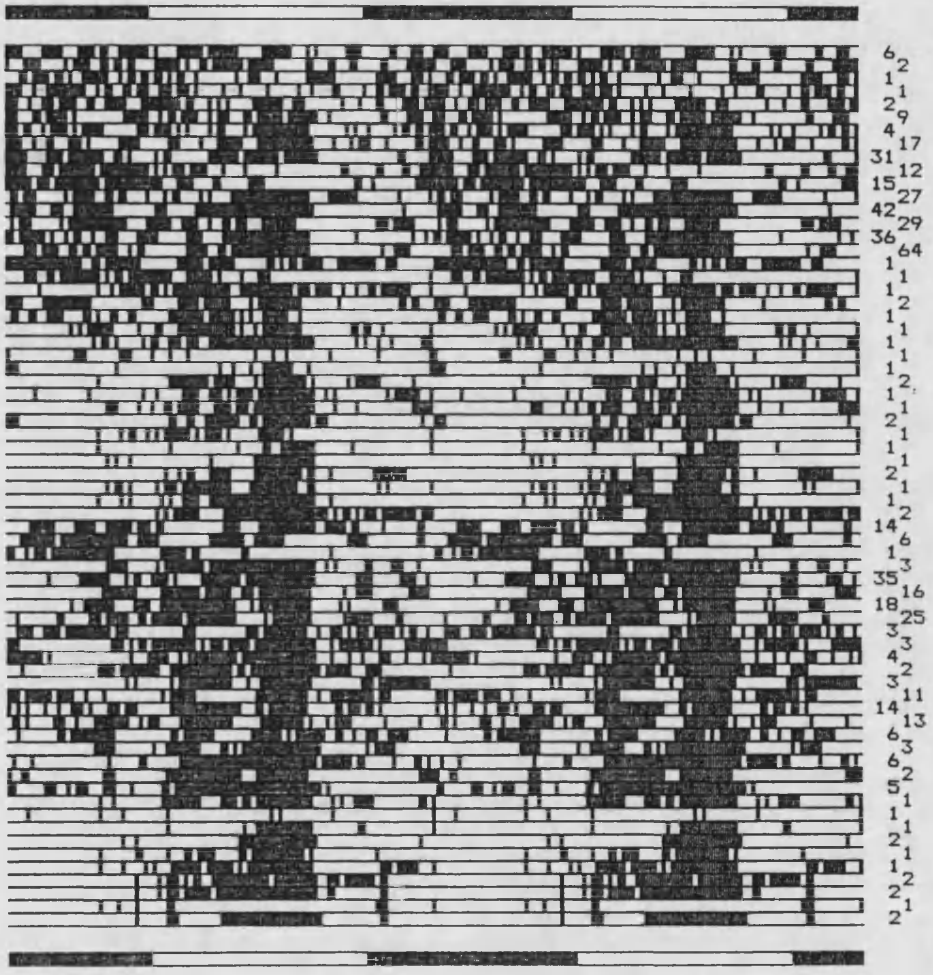


Fig. 5.12b. Effect of melatonin on the phase-shifting ability of light on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 2.

Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram.

DD from day 1

Treatment: 15 min light pulse at 13:00 h (CT12) given on day 28 (animal withdrawn at beginning of day 42).

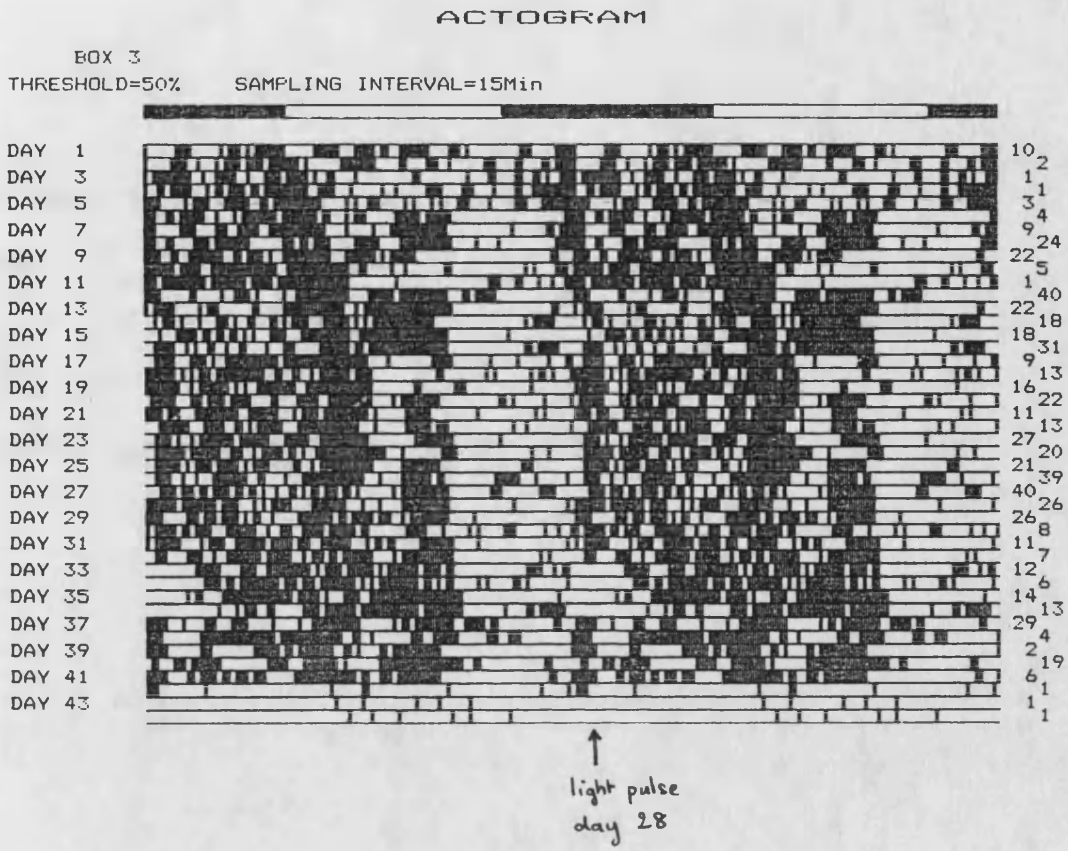

ACTOGRAM
BOX 3
THRESHOLD=50%
SAMPLING INTERVAL=15Min
DAY 1
DAY 3
DAY 5
DAY 7
DAY 9
DAY 11
DAY 13
DAY 15
DAY 17
DAY 19
DAY 21
DAY 23
DAY 25
DAY 27
DAY 29
DAY 31
DAY 33
DAY 35
DAY 37
DAY 39
DAY 41
DAY 43
10 2
1 1
3 4
9 24
22 5
1 40
22 18
18 31
9 13
16 22
11 13
27 20
21 39
40 26
26 8
11 7
12 6
14 13
29 4
2 19
6 1
1 1
light pulse
day 28

Fig. 5.12c. Effect of melatonin on the phase-shifting ability of light on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 3.

Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram.

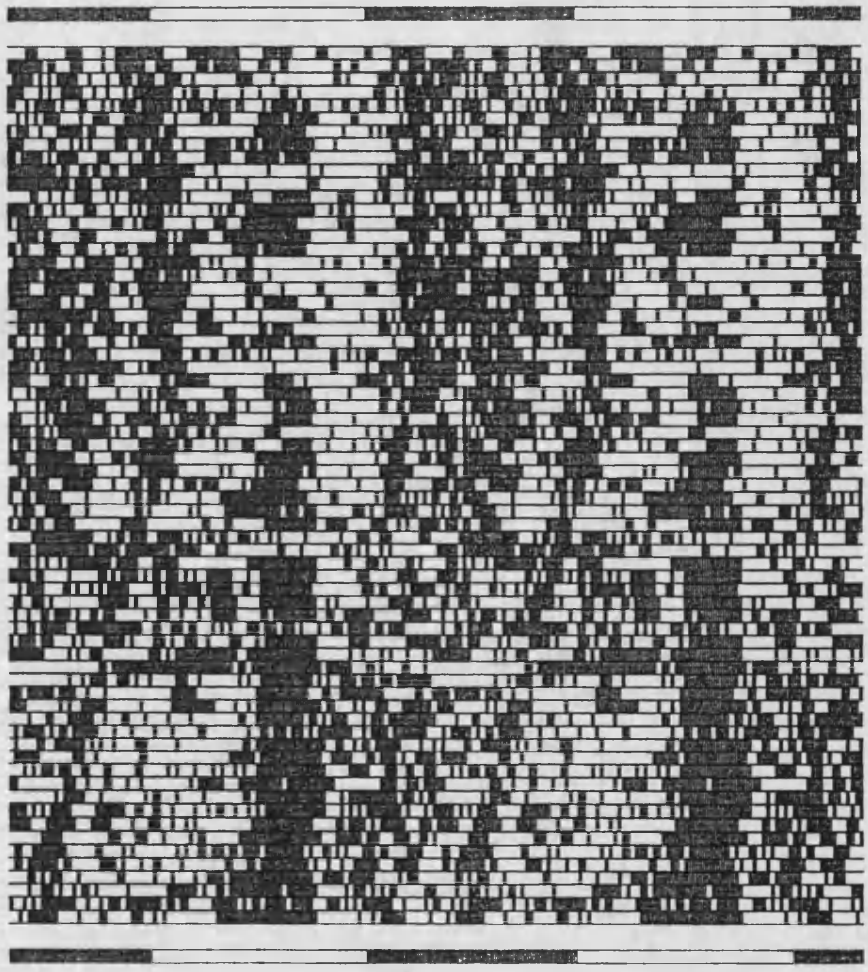
DD from day 1

Treatment: 15 min light pulse at 10:00 h (CT12) given on day 28

ACTOGRAM

BOX 4

THRESHOLD=50% SAMPLING INTERVAL=15Min



light pulse
day 28

Fig. 5.12d. Effect of melatonin on the phase-shifting ability of light on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 4.

Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram.

DD from day 1

Treatment: 15 min light pulse at 16:00 h (CT12) given on day 28

ACTOGRAM

BOX 5

THRESHOLD=50% SAMPLING INTERVAL=15Min

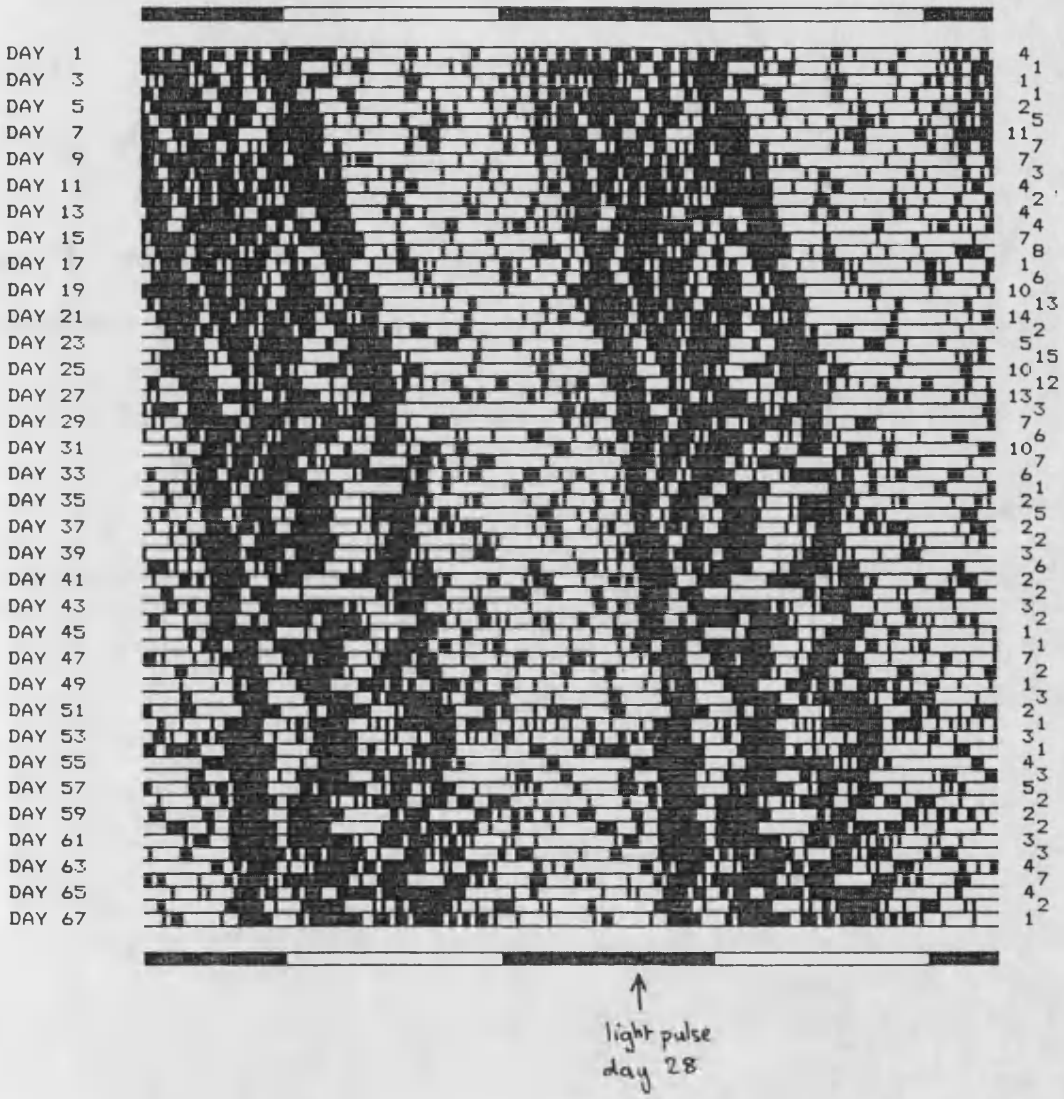


Fig. 5.12e. Effect of melatonin on the phase-shifting ability of light on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 5.

Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram.

DD from day 1

Treatment: 15 min light pulse at 15:00 h (CT12) given on day 28

ACTOGRAM

BOX 6
THRESHOLD=50% SAMPLING INTERVAL=15Min

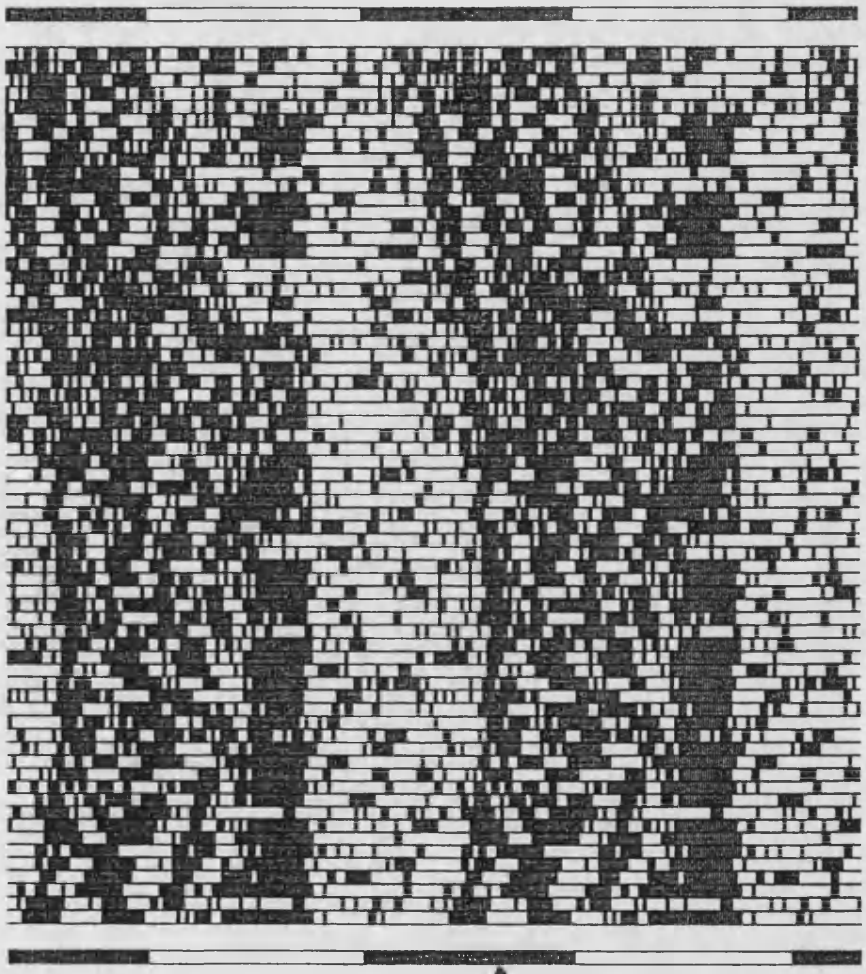


Fig. 5.12f. Effect of melatonin on the phase-shifting ability of light on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 6.
Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram.

DD from day 1

Treatment: 15 min light pulse at 16:15 h (CT12) given on day 28

ACTOGRAM

BOX 7

THRESHOLD=50% SAMPLING INTERVAL=15Min

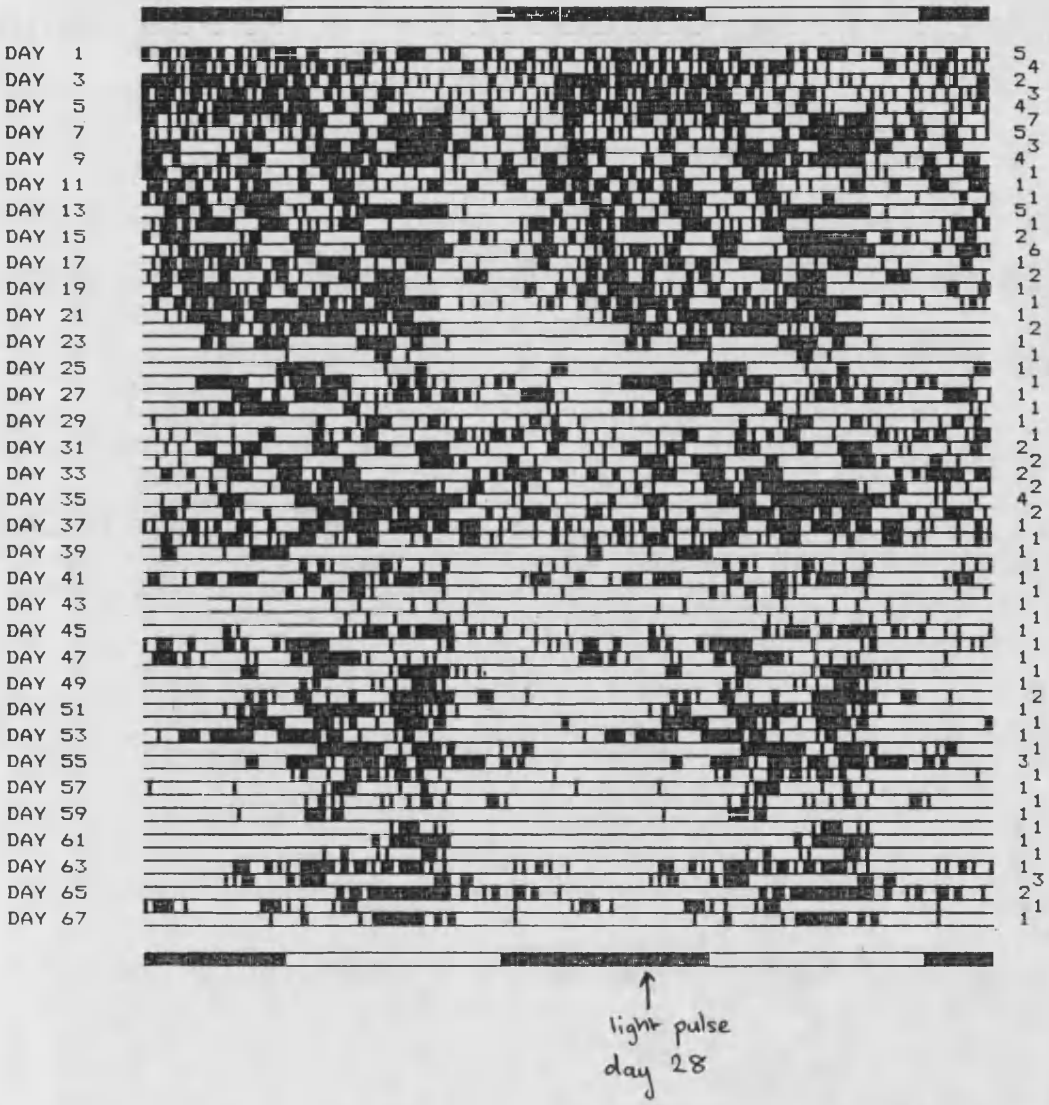


Fig. 5.12g. Effect of melatonin on the phase-shifting ability of light on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 7.

Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram.

DD from day 1

Treatment: 15 min light pulse at 17:15 h (CT12) given on day 28

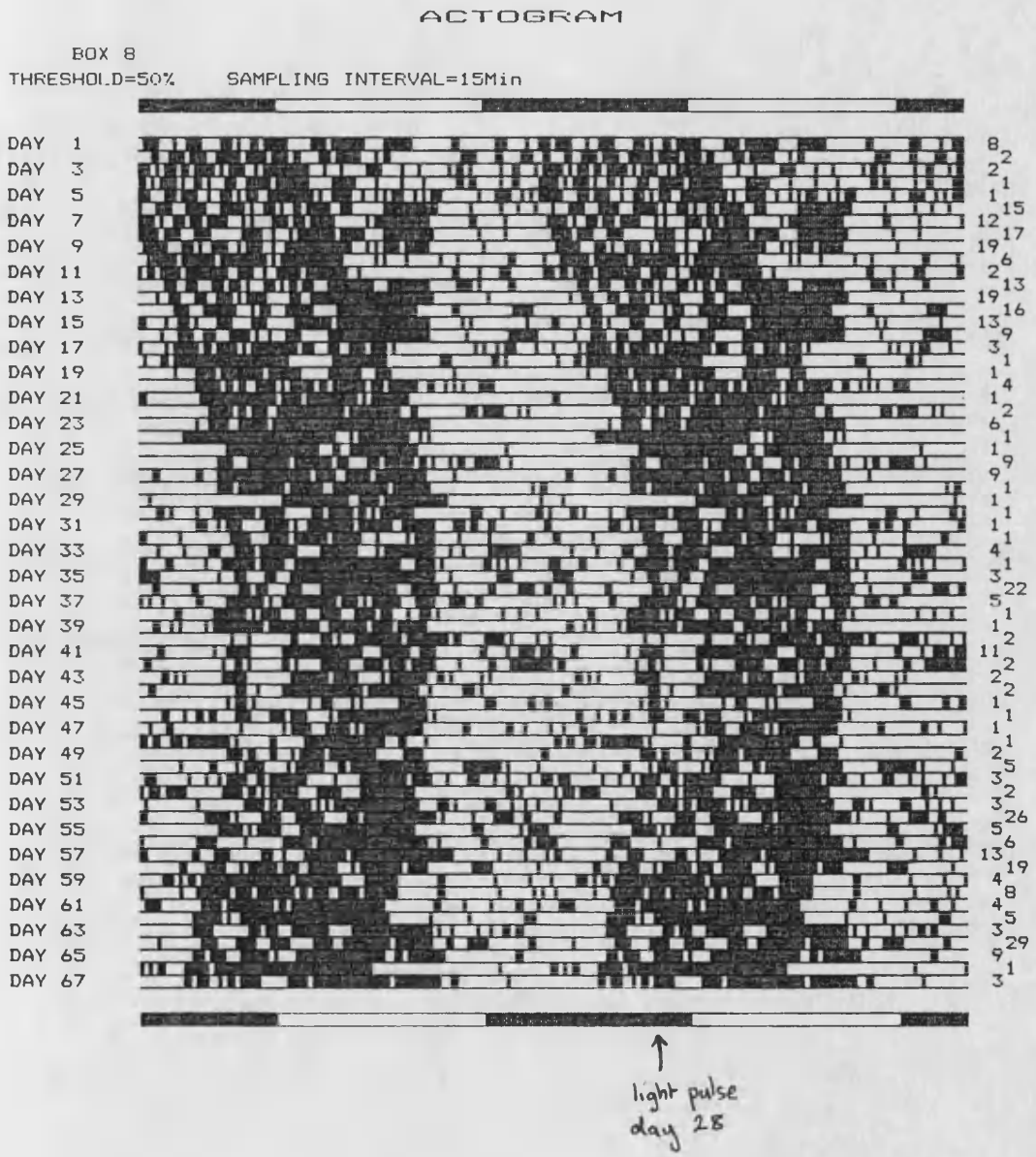

ACTOGRAM
BOX 8
THRESHOLD=50%
SAMPLING INTERVAL=15Min
DAY 1
DAY 3
DAY 5
DAY 7
DAY 9
DAY 11
DAY 13
DAY 15
DAY 17
DAY 19
DAY 21
DAY 23
DAY 25
DAY 27
DAY 29
DAY 31
DAY 33
DAY 35
DAY 37
DAY 39
DAY 41
DAY 43
DAY 45
DAY 47
DAY 49
DAY 51
DAY 53
DAY 55
DAY 57
DAY 59
DAY 61
DAY 63
DAY 65
DAY 67
8 2
2 1
1 15
12 17
19 6
2 13
19 16
13 9
3 1
1 4
1 2
6 1
1 9
9 1
1 1
1 1
4 1
3 1
22
5 1
1 2
11 2
2 2
1 2
1 1
1 1
2 5
3 2
3 26
5 6
13 19
4 8
4 5
3 29
9 1
3
light pulse
day 28

Fig. 5.12h. Effect of melatonin on the phase-shifting ability of light on free-running circadian rhythms: actogram of the circadian rhythm of locomotor activity expressed by rat 8.

Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the daily median threshold printed to the right of the actogram.

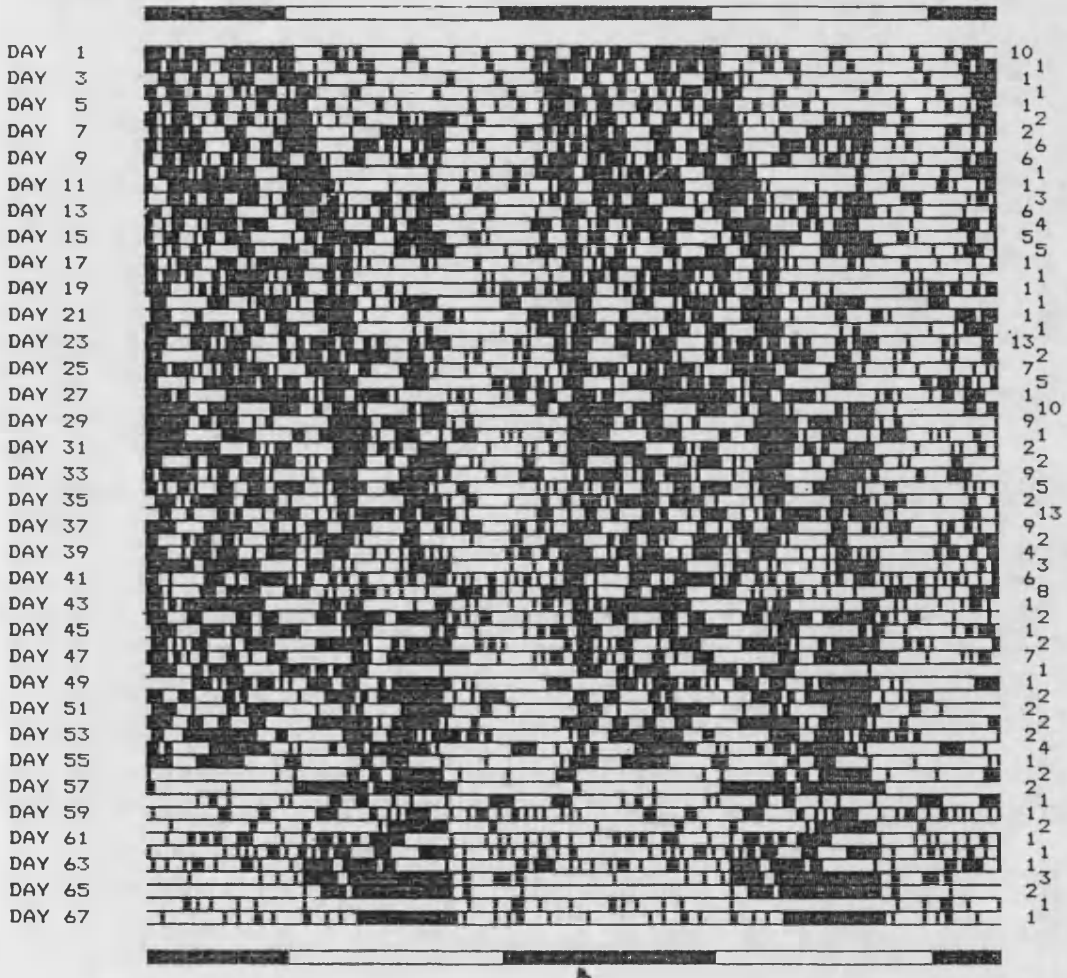
DD from day 1

Treatment: 15 min light pulse at 13:15 h (CT12) given on day 28

ACTOGRAM

BOX 10

THRESHOLD=50% SAMPLING INTERVAL=15Min



5.5.4. Experiment 4: Effect of melatonin on ELA.

Melatonin did not influence ELA in rats at doses up to 50mg/kg at any of the time points investigated (see Fig. 5.13 and Table 5.3).

5.5.5. Experiment 5: Effect of melatonin on SLA.

Melatonin had no significant effect on SLA in rats at doses upto 10mg/kg at either of the two time points investigated (see Table 5.4). Melatonin (1mg/kg) also failed to affect the SLA of rats subjected to a 6 h phase advance of the light or a 6 h phase advance of the dark phase in comparison to saline-treated controls (see Fig. 5.14). It was noted from this work that the sudden change in environmental lighting conditions equilibrated the differing activity levels of MD and ML. Fig. 5.15 shows the melatonin and saline results from each "light" treatment (i.e. phase-shift or no phase-shift) combined.

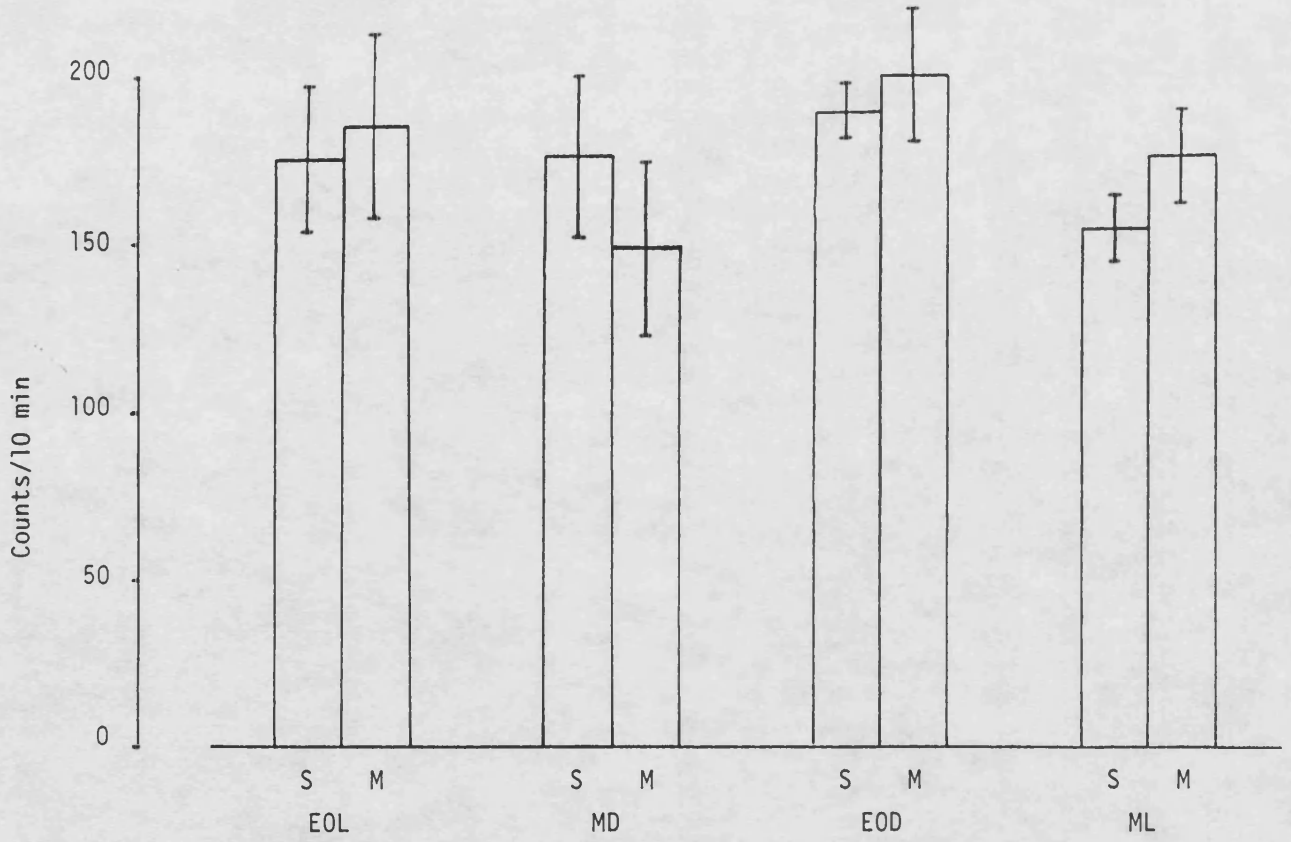
Table 5.3. Effect of melatonin on ELA in rats.

Melatonin Dose (mg/kg)	Pretreatment Time	Time-of-Day	N	Mean Counts in 10 min (+/- s.e.m.)
vehicle	30	ML	5	153 (+/- 10.5)
0.1	30	ML	5	221 (+/- 28)
1	30	ML	6	175 (+/- 13.3)
10	30	ML	5	166 (+/- 30.6)
50	30	ML	5	131 (+/- 22.8)
vehicle	30	MD	6	176 (+/- 25.1)
0.1	30	MD	6	144 (+/- 10)
1	30	MD	6	149 (+/- 29)
10	30	MD	6	185 (+/- 14.7)
50	30	MD	5	204 (+/- 34.3)

Table 5.4. Effect of melatonin on SLA in rats.

Melatonin Dose (mg/kg)	Time-of-day	N	Mean Counts in 2h (+/- s.e.m.)
vehicle	ML	5	78.8 (+/- 23.9)
1	ML	5	79.4 (+/- 22.5)
10	ML	5	64.6 (+/- 10)
vehicle	MD	5	383.2 (+/- 84.1)
1	MD	5	276.6 (+/- 93.6)
10	MD	5	393.4 (+/- 68.2)

Fig. 5.13. Effect of melatonin (1mg/kg) on exploratory locomotor activity in rats over 24 h. (n=5-6, mean ± s.e.m.).

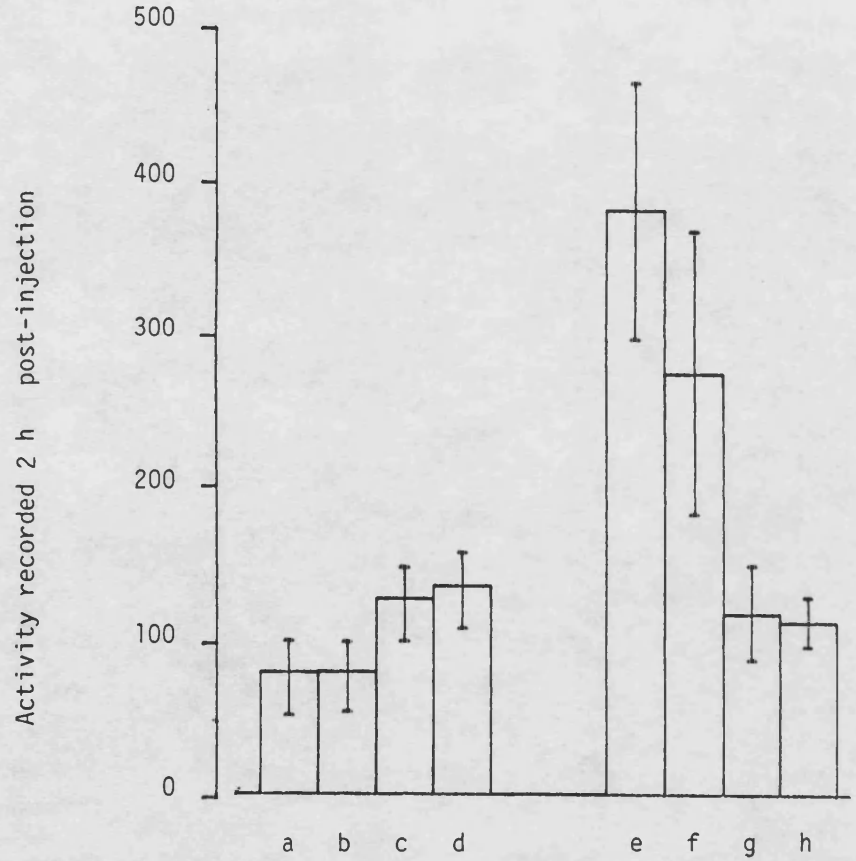


Key:

S = Saline
M = Melatonin
EOL = End-of-light
MD = Mid-dark
EOD = End-of-dark
ML = Mid-light

Pre-treatment time = 10 min

Fig. 5.14. Effect of melatonin (1mg/kg) on the spontaneous locomotor activity of the rat at mid-light and mid-dark and after a 6-hour phase shift. (n=5-6, mean ± s.e.m.)



Key:

a = saline at mid-light

b = melatonin at mid-light

c = saline at mid-light with a 6 h phase advance of the dark phase

d = melatonin at mid-light with a 6 h phase advance of the dark phase

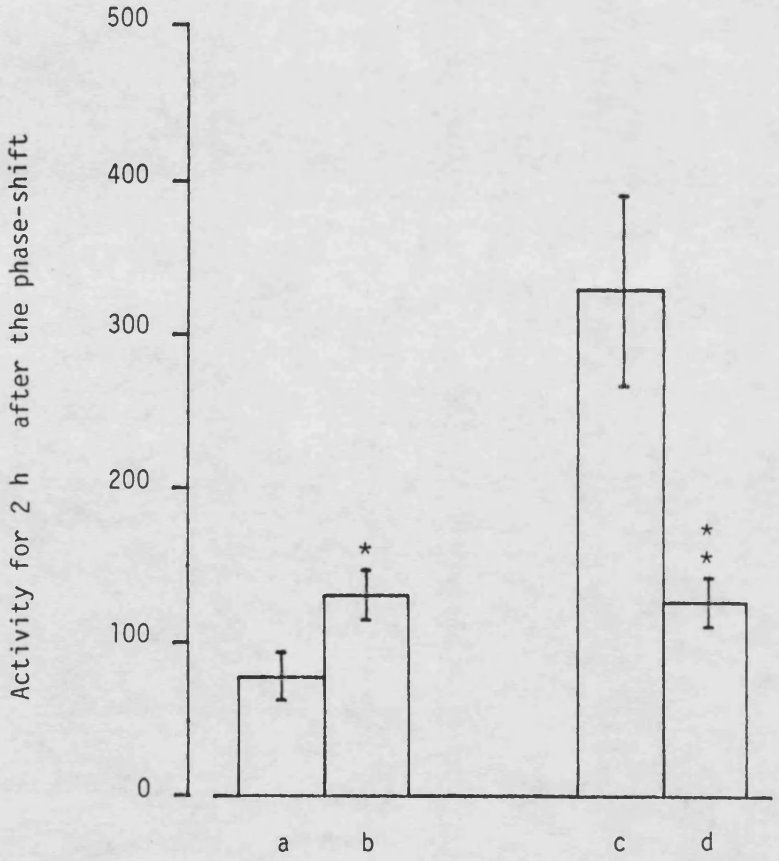
e = saline at mid-dark

f = melatonin at mid-dark

g = saline at mid-dark with a 6 h phase advance of the light phase

h = melatonin at mid-dark with a 6 h phase advance of the light phase

Fig. 5.15. Effect of a 6-hr phase shift of the LD cycle on spontaneous locomotor activity in the rat. (n = 5-10, mean ± s.e.m.)



Key:

a = activity at mid-light

b = activity after a 6 h phase advance of the dark phase

c = activity at mid-dark

d = activity after a 6 h phase advance of the light phase

* p.< 0.05 (t-test)

** p.< 0.01 (t-test)

5.6. Discussion

The results generated by this series of experiments concur with previous published work on the effect of melatonin on the circadian rhythm of locomotor activity (e.g. Redman et al., 1983). Melatonin is unable to affect the rate or direction of re-entrainment of the locomotor activity circadian rhythm following a 180° phase-shift of the LD cycle. The protocol used in assessing the effect of melatonin on the rate of entrainment depended on dosing the animals prior to the phase-shift at the end of their current activity phase (dark phase). Thus plasma levels of melatonin would have been approaching daytime (i.e. negligible) amounts. It was considered a reasonable hypothesis that a sudden unexpected peak of melatonin might condition the biological clock of the animal to adjust to levels more appropriate to dark-onset. The subsequent 12 h phase-advance of the scotoperiod meant that this time of injection corresponded to the onset of the dark phase. In retrospect this may not have been the most appropriate procedure to adopt. Assuming that the SCN of the hypothalamus is the loci of action for melatonin with regard to its effects on the expression of circadian rhythms, putative melatonin receptors in this region have been shown to undergo a circadian rhythm such that their density is greatest during the transition period from light to dark (Vanecek et al., 1988) and least during the transition from dark to light. The reduction in receptor density during the latter half of the scotoperiod is thought to be initiated by melatonin itself (Reiter et al., 1978). This is thought to explain the effectiveness of late afternoon injections of melatonin in eliciting gonadal regression rather than at other times (Tamarkin et al., 1976; Glass and Lynch, 1982; Vanecek et al., 1988). Despite no apparent change in binding

site affinity, an increase of receptor number would increase the sensitivity of the hypothalamus to the presence of melatonin. Therefore, although melatonin was given at the forthcoming sensitive stage of the melatonin receptor rhythm the timing of initial injections fell upon the least responsive stage. It is not known how long it takes for the circadian rhythm of melatonin receptor density to re-entrain to a new cycle. Presumably it might take as long as other physiological cycles, e.g. temperature, adrenocortical rhythm, in which case 14-21 days is often taken as a safe period to allow the establishment of equilibrium. Thus it is possible that the injections of melatonin were being given at the least appropriate time throughout the critical stage of the experiment. The assumption made at the beginning of the experiment was that the presence of melatonin in the plasma at an atypical time would be registered in some way by the organism thereby rendering it more or less adaptable to a phase-shift. This does not appear to have happened. The successful strategy adopted by Arendt and colleagues (Arendt et al., 1986) in their jet-lag experiments with human subjects involved synchronizing the pulse of exogenous melatonin to the responsive phase of the initial LD cycle and then changing the time of injection to correspond to the new responsive phase. It is possible that the exposure to exogenous melatonin caused a premature down-regulation of hypothalamic melatonin receptors. This induced a phase-advance of the following period of high melatonin receptor sensitivity leading to those physiological cycles driven by the hypothalamus to also shift. Interestingly, simulated jet-lag studies have reported less success using melatonin though the equivalence of phase-shifts using subject isolation techniques with genuine time-zone transition methods has been questioned (Wever et al.,

1986; Arendt, 1988). The most difficult question to answer concerning jet-lag is whether or not the phase-advancing effects of melatonin are genuine reflections of the master oscillator. By monitoring several circadian parameters Arendt et al. (1987) endeavoured to circumvent this problem. They demonstrated more rapid re-entrainment of the adrenocortical rhythm and of the endogenous melatonin rhythm itself in subjects given melatonin as opposed to placebo (there is no evidence as yet to suppose that melatonin influences its own synthesis by an action other than indirectly through the circadian pacemaker). Additionally, results from psychological tests and sleep data suggested that these subjects adjusted more rapidly and endured less jet-lag symptoms from the shift than those that received placebo. This, however, may not necessarily be attributed to an effect on the pacemaker. The phenomenon of masking may account for the results. This is said to occur when the true phase of a circadian cycle, as dictated by the actual phase of the pacemaker, is obscured by an action of endogenous or exogenous orign that affects the apparent phase-position of the overt cycle but not the underlying oscillator-controlled rhythm. For example, locomotor activity (the visible rhythmic characteristic) can be suppressed by a sedative (e.g. haloperidol) and body temperature is affected by activity. Melatonin has been reported as having a suppressive effect on the adrenal glands and cortisol production (Vaughan et al., 1972; Ogle and Kitay, 1977; Relkin et al., 1983), though this is disputed since Waldhauser et al. (1984b) found that melatonin induced no response in human serum cortisol levels. This latter result may be more pertinent since the earlier studies used rodents. Melatonin also exhibits a sleep-promoting action which is believed to be a

pharmacological rather than a physiological property though this has yet to be proven (see Leiberman and Lea, 1988). For practical purposes, however, "masking" may well be a phenomenon that can be ignored if melatonin improves the symptoms of the jet-lagged passenger without the induction of the "hangover" attributed to the benzodiazepine hypnotics that also evince phase-shifting properties, e.g. triazolam (Turek and Losee-Olson, 1986; Turek and Van Reeth, 1988; Van Reeth and Turek, 1989a; Van Reeth and Turek, 1989b).

Unfortunately a comprehensive and systematic approach to the study of the effect of melatonin on entrainment has yet to be conducted. Several questions remain unanswered. For example, assuming that the effects of melatonin on jet-lag are the result of an action on the biological clock can melatonin only increase the rate of entrainment? Would continued treatment with melatonin at the old responsive time reduce the rate of entrainment? Would melatonin affect the rate of re-entrainment for a rhythm coupled to a zeitgeber other than light? And, ultimately, is melatonin affecting the "hands" or the "clock" mechanism of the master oscillator?

A further observation made in this series of experiments is that all animals answered the new phase position of the LD cycle by adjusting the onset of their activity period with sequential phase-delays until synchrony had been re-established. It is possible that the direction, in this situation which concerned a reversal of the LD cycle, had been dictated by the 24 h of darkness and the experiment is therefore worth repeating using 24 h of light to effect the 180° phase-shift.

Melatonin was shown to entrain a free-running locomotor activity rhythm if given at a time which coincided with the approximate onset of activity (CT12). To effect such a response

melatonin essentially performed small phase-advances. Cessation of melatonin treatment tended to return the cycle to its previous free-running state. This did not occur in every case, however, and also one saline-treated rat appeared to entrain to the injection. The first set of results (see Figs. 5.9a-5.9d, corresponding to rats 1-4) were generated using equipment far more sensitive to activity than the second set which used light beam breakages (Figs. 5.9e-5.9j, corresponding to rats 5-10). This sensitivity is clearly demonstrated by comparison of the median levels of each day's activity printed on the right of the actogram. Due to this change in activity recording equipment, comparison of the total daily activity between melatonin and saline-treated animals cannot be made. The first set of data is difficult to evaluate because entrainment has persisted following the last injection of drug or placebo in two of the animals (rats 1-4). Some unidentified environmental factor is probably responsible for this effect. In the entrained state the epocrepuscular nature of the animals appears to be accentuated, which may be partly due to an extended period of activity in comparison to the rest phase. It may also be explained by a differential effect on the "morning" and "evening" oscillators of Pittendrigh and Daan's "dual oscillator" hypothesis for the physiological control of circadian rhythms (Pittendrigh and Daan, 1976). The clear free-running rhythm expressed by the rat in Fig. 5.9a demonstrates the potential source of confusion resulting from stress-induced activity which occurs upon the administration of the drug and the return of the animal to its cage. Both the onset and offset of the activity phase should be monitored for a more reliable picture with regards to the effect of a given treatment. Complicating the proceedings further is the suggestion by Armstrong

(1989) that, because of the "narrow window of sensitivity" in the circadian phase of the pacemaker during which melatonin can act as a zeitgeber, if the free-running period is too great entrainment may not occur.

The low level of data generated by the second set of animals (see daily median values of Figs. 5.9e-5.9j) ruled out the use of daily activity plots as a means of analysing the onset and offset of activity. This also had an adverse effect on the resolution and accuracy of the periodogram (compare Figs. 5.10a-5.10d with Figs. 5.10e-5.10i). Inspection of the actograms reveals that at least one animal entrained both the onset and the offset of its activity cycle to melatonin treatment (see Fig. 5.9e). However, its free-running period post-treatment appears to be much reduced in comparison to its period prior to the administration of melatonin. The periodogram estimates the period for days 4-13 (pre-melatonin treatment) as being 24 hrs, for days 17-26 (during melatonin treatment) it is 23.75 hrs and for days 29-38 (post-melatonin treatment) it is recorded as 24.25 hrs. Additionally, the graphs depicting the fundamental period and its harmonics are clearly less well resolved in comparison to those referring to the earlier set of entrainment experiments (note scale on ordinate axis of Figs. 5.10a-5.10d and Fig. 5.10e). It would appear that, comparing visual inspection of actogram data and periodogram analysis, the latter technique tends to underestimate the apparent period. Figs. 5.11a and 5.11b and Table 5.1 depict the period derived from periodogram analysis using a twenty day interval. This could only be obtained before and after treatment for rats 1 to 4 since the time schedule of the subsequent experiment was not long enough. A more reliable approximation of the period is obtained with

improved resolution of the fundamental. However, over the same time intervals, there remains some discrepancy between the periodogram calculated estimate of the period and that obtained by simply drawing an eye-fitted line on the actogram that approximates successive onset of activity times and measuring the gradient (see Tables 5.1 and 5.2).

An additional problem which concerns the reliability of the data generated by animals 5-10 is the emergence of "foreign counts". These are most clear on Fig. 5.9f since the animal was actually withdrawn from the experiment at the end of day 23. Thus no activity should have been registered. Interference from an unknown electrical source probably accounts for the thin vertical lines prominent in Figs. 5.9e, 5.9g, 5.9h, 5.9i, and 5.9j. In these figures the trend of locomotor activity is still clearly discernible but the severity of the malfunction increased during the subsequent experiment designed to investigate the effect of melatonin on a light-induced phase-shift of the circadian rhythm in locomotor activity. Figs. 5.12a to 5.12c and 5.12e to 5.12h which relate to this experiment show thick vertical bands of putative activity. This problem remained insuperable despite the dismantling and rebuilding of the equipment, and changing the interior design of the environmental cabinets which allowed the photoreceivers and phototransmitters to be mounted closer together (a means of increasing the signal-to-noise ratio). A current dampener was also employed on the power lines to the computer and interface to no avail. Despite this it is possible to distinguish trends in the activity profile of a particular animal using the actogram. When given a 15 min light pulse at CT12 on day 28 of the experiment only one animal appears to have responded with the expected phase-delay

(see Fig. 3) which actually manifested as a series of phase-delays; subsequent ill-health forced its early withdrawal. The responses made by the rest, that can be reasonably distinguished, were a shortening of the free-running period even to the extent that it became, in at least two cases (Figs. j and k), shorter than 24 h (as shown by an actogram with an onset of activity time gradient running right to left). Reasons for the dichotomous nature of the results following identical treatment is not clear. It is known that the shape of a light-induced PRC is a function of the period which varies between individual animals (Daan and Pittendrigh, 1976). There is also variation of the shape of the PRC according to the species under study (Honma et al., 1978; Daan and Pittendrigh, 1976). However, a single light pulse used to elicit a phase-shift should not also evoke a lasting change in period. Following recovery from the shift the original period should have been restored.

Time restrictions, caused by endeavours to overcome the problem of "cross-talking" (counts recorded by the breakage of one light beam being registered by the computer as if two or more beams had been broken) and the registering of foreign counts, was a major limiting factor in this experiment. Eventually only one time point could be chosen for investigation. CT12 is on the borderline of the subjective day in a nocturnal rodent such as the rat. This point also corresponds to a phase position that is most receptive to a pulse of melatonin and follows soon after the "dead zone" of responsiveness to light (Daan and Pittendrigh, 1976). However, using a phase point more responsive to light would have been to risk losing the sensitivity to melatonin if it was to possess an observable effect. Clearly a comprehensive answer to the original question of whether melatonin can influence a light-induced

phase-shift requires more time and equipment less subject to spurious counts. Ideal conditions would have allowed the construction of a 15 min light pulse-induced PRC followed by its repeat using bolus doses of melatonin/placebo as pretreatment.

Exploratory locomotor activity was not affected by melatonin and did not exhibit a circadian rhythm. Exploration is a fundamental aspect of animal behaviour and is described by Silverman (1978) as "information-seeking independent of associated motor activity". The strength of motivation in assessing a novel environment for food and drink sources, danger and escape routes provides enough impetus to override or bypass any dampening effect on locomotor activity the biological clock might exert. This is borne out when a comparison with spontaneous locomotor activity is made. Although only two time points were investigated there is a clear difference between the level of locomotor activity expressed at ML in comparison to MD. The lack of effect of melatonin in both paradigms would suggest that it not only exerts no influence on locomotor activity at the time points investigated but also that it lacks influence on exploratory drive, though further verification with regard to this behavioural parameter was beyond the scope of this thesis.

The most striking result obtained during the investigation of short-term locomotor activity was the influence that a 6 h phase-advance of the light or dark phase had on SLA. Either treatment resulted in an equilibration of activity, regardless of whether the animals received saline or melatonin. Thus light suppressed activity and darkness evoked activity though not to the extremes that would be expected according to the appropriate phase. This reaction is most likely to be a consequence of adaptive

behaviour rather than a direct action on the biological pacemaker. For example, using an anthropomorphic analogy, a well-known lighted environment becomes almost novel to a human suddenly plunged into darkness and would suppress activity until acclimatization to the surroundings had been achieved. Rats are known to favour a dark environment to a well-lit one. It might therefore be expected for a rat to explore the vicinity after extinguishing the lights which would be detected as ELA rather than SLA. Conversely, rat locomotor activity would be suppressed following the sudden illumination of a darkened cage. The result highlights the problem in correctly evaluating the origin of an overt behavioural response elicited when, in this instance under entrained conditions, a sudden change in the environmental conditions is imposed. Analysis of the pattern of locomotor activity using actograms may fail to reveal a simple direct effect on animal behaviour rather than on the underlying circadian rhythm. However, it should be said that this change in behaviour may itself subsequently affect the major oscillator. For example, Van Reeth and Turek (1989a) found that by forcibly restricting the activity of hamsters the phase-shifting ability of dark pulses and the benzodiazepine, triazolam, can be inhibited. The conclusion, though, that their results bring into question the current hypotheses, that dark pulses and certain drugs influence the neural pathways that mediate environmental LD information, may be rather parochial. It is more probable that a variety of systems of differing heirarchy are involved.

CHAPTER 6.

LIGHT/DARK DISCRIMINATION

6. LIGHT/DARK DISCRIMINATION

6.1. Introduction

Light is acknowledged as the most important zeitgeber of circadian rhythms in mammals. The relationship of light to melatonin secretion was first established by the work of Wurtman et al. (1963, 1964). Constant light (LL) suppresses melatonin synthesis and hence the rhythm, though the extent of suppression varies according to the intensity of light employed and the species concerned (Lewy et al., 1980; Reiter, 1985). The rhythmic production of melatonin persists in constant darkness (Ralph et al., 1971), indicating its authenticity as a circadian rhythm. Short pulses of light of appropriate intensity can phase-advance or phase-delay melatonin production according to the time of the pulse in relation to the phase of the LD cycle (Czeisler et al., 1986; Broadway et al., 1987; Lewy et al., 1987). Melatonin itself has been shown to induce phase-advances of nocturnal melatonin secretion if given towards the end of the normal light phase (Arendt et al., 1985). The LD cycle has a third effect on the rhythm of melatonin production. As day-length increases, the duration of the melatonin signal is reduced (see Rollag and Niswender, 1976), i.e. melatonin secretion exhibits photoperiodism, the implications of which have been discussed in chapter 1. To briefly recapitulate, precisely-timed manipulation of plasma melatonin levels can elicit short-day (i.e. winter) appropriate responses in susceptible species when the environmental LD cycle directs otherwise. Such responses can be in the form of advancing the oestrus cycle in short-day breeders (e.g. ewes) or inducing winter coat growth in mink. The essential feature of these responses is that the biological processes controlling them

under normal circumstances have been deceived, by the presence of melatonin, into behaving in a manner inappropriate to external conditions. This interpretation can be applied to the work of Redman et al. (1983) who demonstrated the ability of exogenous melatonin to entrain free-running locomotor activity rhythms of rats. The time of activity onset of the animals studied by Redman and co-workers occurred slightly later each day (indicating a period greater than 24 h). However, when the injection of melatonin coincided with this time entrainment took place. The implication of this is that the presence of melatonin, or the rapid rise of melatonin plasma levels, indicated to the rodents the onset of subjective night. Earlier work by Gwinner and Benzinger (1978) showed that pinealectomized European starlings, normally diurnally active, synchronized their activity rhythms after melatonin injection at the end of their active phase. The capacity for melatonin to elicit what would appear to be a general co-ordinating signal for circadian rhythms initiated the following investigation into the degree of awareness that the animal had of this effect. This question has already been partially addressed in the previous chapters, but it was thought that a more direct approach might yield more promising results. In essence, the hypothesis which the investigation hoped to prove or disprove was whether melatonin, as the "darkness hormone", might change light-appropriate behaviour into dark-appropriate behaviour.

Animal behaviour can be split chronologically into two fundamental components - that which is expressed during the dark phase and that expressed during the light phase of a LD cycle, e.g. rats are active and consume most of their 24 h input of food and drink in the scotoperiod while rest is confined to the light phase. For diurnal animals the reverse is true. If melatonin caused animals

to express behaviour normally associated with darkness then it would be expected to increase spontaneous activity and food consumption in the light phase. Additionally, since the number of headtwitches induced by certain serotonergic agonists is maximal at ML, pretreatment with melatonin might be expected to reduce that number to a level associated with MD. The results from experiments conducted in the preceding chapters (3 and 5) indicate that melatonin elicits no such effect. This may be due to the insensitivity of target receptors for melatonin which would appear to be responsive only at the end of the light phase (Reiter, 1983b, Armstrong, 1989; Zisapel et al, 1988). However, it can be argued that the type of behaviours being investigated in these experiments is not suitable for resolving the hypothesis. For example, mCPP-induced hypoactivity in rodents has been interpreted as not being due to a direct action on the biological clock but rather derived from an action on 5-HT$_{1c}$ receptors (Kennett and Curzon, 1988a). 8-OHDPAT-induced hyperphagia is attributed to an effect on central (Hutson et al., 1986; Bendotti and Saminin, 1986) and, possibly, peripheral (Sleight et al., 1988) serotonergic components of the feeding mechanism. It must be said, though, that these compounds have yet to be subjected to long-term studies to investigate potential effects on the circadian rhythms of locomotor activity and feeding.

It was considered that drug discrimination might prove a more suitable behavioural technique to investigate whether melatonin can affect an animal's interpretation of an exteroceptive stimulus. Data previously obtained from the drug discrimination studies described in chapter 3 suggest that melatonin, on its own, fails to affect cognition. However, it seemed possible that animals trained

to discriminate the exteroceptive stimuli of light and dark, in certain circumstances might be prompted to make a dark-appropriate response if given melatonin during a "light" session. The use of exteroceptive stimuli precedes drug-induced interoceptive cues in discrimination experiments though this area tends to concentrate on pattern and colour recognition. More recently Jarbe and co-workers have initiated a series of studies investigating the interaction between drug discriminative stimuli and exteroceptive sensory signals (Jarbe et al., 1981; Jarbe et al., 1983; Jarbe and Johansson, 1984). These experiments, however, examined the effects of pairing exteroceptive stimuli with drug cues during training. Jarbe and Johansson (1984), for instance, were able to show that the exteroceptive stimuli of light and dark provided a stronger discriminative cue than 10mg/kg of pentobarbital. Thus animals were trained to respond on one lever in the presence of light and drug and on the other lever in the presence of dark and saline. Under test conditions of dark and drug or light and saline the subjects chose the lever appropriate to the external evironmental conditions. Sound (in the form of a low and high frequency tone), paired with 10mg/kg of pentobarbital, had no ability to influence the interoceptive stimulus provided by the drug. Closer to the experimental work described in this chapter, and independent of Jarbe, Koek and Slangen (1984) reported the effects of various centrally-active agents on the discriminative ability of rats using visual and tactile stimuli. In this study training was characterized by a grid or wooden floor being paired with an illuminated or darkened conditioning chamber. Drugs were then administered to examine their effects on accurate responding. Cross-testing between the external stimuli was also carried out, i.e. an animal trained to

differentiate between an illuminated chamber paired with a wooden floor from a darkened chamber paired with a grid floor was tested in a darkened chamber with a wooden floor. The results suggested that in this situation individual component stimuli controlled responding equally in most rats of the group.

6.2. Materials and Methods

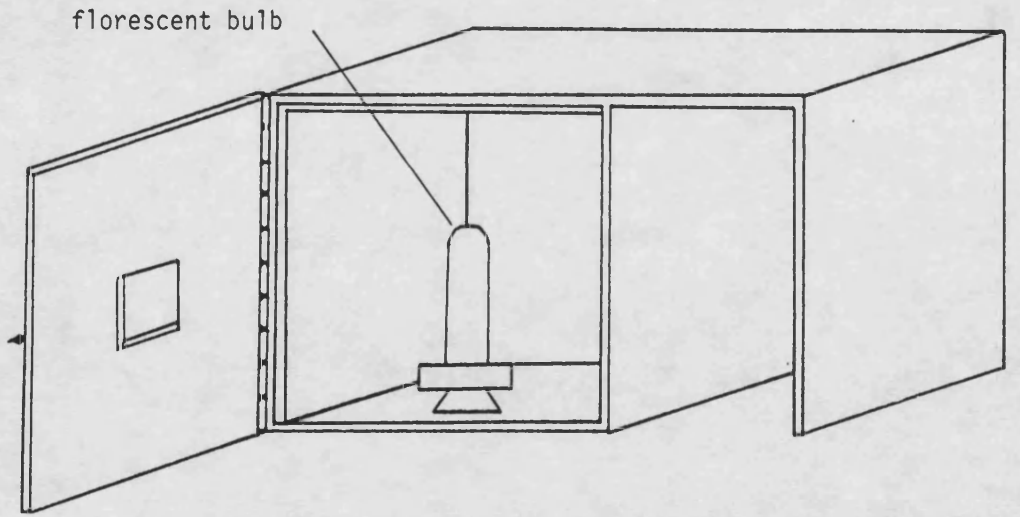
6.2.1. Drugs

Melatonin (Sigma), 1mg/kg, was dissolved in normal saline by sonication. Melatonin, 10mg/kg, was suspended in 0.1% w/w tragacanth in normal saline. Control animals were given vehicle. All injections were given i.p. using a dose volume of 5ml/kg.

6.2.2. Apparatus

Standard operant conditioning chambers containing two levers were used (Campden instruments). Liquid food (0.02ml, 1:1 sweetened condensed milk:water) was delivered via a motorized dipper from a food reservoir located below (see Fig. 4.1, chapter 4). The reinforcement delivery chamber was situated midway between the levers. Presentation of the food and recording of behavioural responses was achieved using a BBC Master computer and software designed and written by the author (for details see Appendix). Control of the lighting within the interior of the Skinner box was achieved by the construction of wooden lighting cabinets (see Fig. 6.1) which fitted snugly in front of the opening to the Skinner box chambers. An 11W fluorescent bulb was used as a source of white light which provided 600 lux as measured in the operant conditioning chamber. Light intensity within the chamber when the fluorescent bulb of the lighting cabinet was off and the experimental room light on was <1 lux.

Fig. 6.1. Diagram of lighting chamber used in the light-dark discrimination studies.



6.2.3. Animals

8 Male Wistar rats (Animal House, University of Bath strain) matched for age and weighing between 150 and 200g at the start of the study were housed in groups of 4 in standard plastic cages (520 x 350 x 170mm). The animals were maintained on a 12:12 LD cycle (lights on 05:00 h) with light intensity during the photoperiod being 200lux (measured at cage height; Macam Photometrics Ltd., Radiometer/Photometer R101). Dim red light (<1 lux, measured at cage height) was provided during the dark phase. Water was provided *ad libitum* and food was made available according to the requirements of the experiment with at least one continuous hour of feeding provided provided in any 24 h period. Animals were examined daily for signs of ill-health and their weight was monitored weekly.

6.2.4. Training Procedure

Rats were gradually acclimatized to a restricted diet schedule which initially comprised a 2 h food presentation immediately following their training session. Once lever-pressing had been established (using continuous reinforcement training conditions during the light phase), training sessions lasting 15 min were introduced. The criterion for reward was gradually increased until the animals were responding satisfactorily on a FR20 schedule. At this point light-dark discrimination training began in earnest with each session limited to 15 min, the provision of food being dictated by the training schedule. In order to overcome the possible cue properties of an aversive i.p. injection given during future experimental sessions, rats were pretreated with saline, i.p., 30 min before the start of a training session.

Clearly, animals could not simply be trained to discriminate between light and dark *per se* since this does not take into account the change in behaviour associated with the change in phase from light to dark. The difference in behaviour expressed during the dark phase and the light phase was considered to be as important as the environmental stimulus in determining the discriminative cue. Therefore, training sessions were designed to accommodate the time of day. Due to the ease of use and the greater wealth of data that it yields the Skinner box paradigm was chosen as the technique for conducting this experiment (for a diagram of this apparatus see chapter 4, Fig. 4.1). A 12:12 LD cycle (with the lights on time of 05:00 h) was chosen so that training sessions could be conducted at any time between 09:00 h and 01:00 h. Thus in any given 24 h cycle there were 8 h of light followed by 8 h of dark during which training sessions could be carried out. The same randomized sequence of training sessions used for the drug discrimination experiments was employed in this study. To avoid light and dark becoming the dominant discriminative stimuli the number of training sessions performed during any 16 h period varied between one and three up to a maximum of two in a row in any particular phase. That is, during one 24 h cycle there might be two "light" training sessions followed by one "dark" training session, while on a subsequent day there may be only one "dark" training session. Animals were usually fed immediately after the last training session of the day unless a "light" training session was planned for the following day. In this instance, food was withheld until after that session. As expected, rats quickly learnt to feed as soon as nourishment was provided. It was also found that food given before midday did not affect a "dark" training session. One

rat was forced to be withdrawn during the course of the study through ill-health but subsequent recovery allowed it to be reinstated following re-training.

Positional preferences were controlled for by training half the animals to press on the right lever in response to darkness and the other half the left lever. To prevent odour cues from influencing choice the levers were wiped with alcohol prior to each session with an animal (Extance and Goudie, 1981). The threshold for criterion, when animals could be used for experiments, was set at 80%, that is 8 correct choices in 10 consecutive sessions.

6.2.5. Experimental Procedure

Training sessions were conducted on weekdays until a satisfactory response rate had been attained. Experimental sessions were then introduced and performed on Tuesdays and Fridays with the remainder of the week reserved for continued training. Experimental sessions were 5 min long during which either lever if pressed 20 times would provide reinforcement. The first lever to receive 20 presses was designated the chosen lever and became the reinforcement lever. Further responses on the other lever were recorded but had no programmed consequences. If the performance of a rat dropped below the criterion level during training it was not used in experiments until the appropriate standard had returned. To prevent animals being able to differentiate between experimental and training sessions, after each experimental session subjects were placed in a "neutral" plastic cage for the 10 min remaining of the 15 min associated with a training session rather than being directly returned to the home environment.

The first series of experiments involved animals being

given melatonin (0, 1, or 10mg/kg) 30 min before a test session, which lasted 60 min.These sessions were timed to begin 60 min before the ML, EOL, MD and EOD phases of the LD cycle. The lighting conditions in the operant conditioning chamber corresponded to the appropriate time. In the second series of experiments melatonin (0, 1 or 10mg/kg) was administered to the animals again 30 min before the start of a test session which was conducted at ML or MD but this time the lighting conditions in the experimental chamber were reversed. Thus, an animal taken from the home cage at ML was placed in a darkened experimental chamber. This, in essence, was a "temporary" 6 h phase advance of the dark phase. The final series of experiments involved pretreating the animals with 30 min of the opposite environmental condition to what time dictated in addition to receiving saline or melatonin (1mg/kg) before being placed in the chamber which continued this condition. Thus an animal taken from the home cage at MD was put into a standard plastic cage (420 x 260 x 150mm) housed in an environmental cabinet (see chapter 5, Fig. 5.1) and given 30 min of light (850 lux) before being tested in the illuminated operant conditioning chamber. Animals used at ML were given the opposite treatment. The experimental room was maintained according to the normal LD schedule such that immediately after an experimental session the rat was returned to its pre-experimental light or dark phase. At least 72 h separated experimental sessions involving a change in phase.

6.3. Data Analysis

Discrimination during training was expressed as a percentage of correct choices in 5 consecutive sessions. Three parameters were used as measures of performance during training sessions: the FRF value (ratio of correct lever presses against incorrect lever presses until the FR number is reached on the reinforcement lever expressed as a percentage of the total), the response latency (time taken to gain the first reinforcement), and the response rate (rate of correct lever presses after the first reinforcement until the end of the session). These parameters were only used if an animal had pressed a lever a sufficient number of times to gain at least one reinforcement. For training sessions the response rate parameter and the FRF value have been represented as the mean of 5 consecutive sessions from those rats that pressed the lever sufficiently to gain at least one reinforcement. During lever press training it was found that some animals tended to press the levers almost as soon as they were put into the chamber. It was therefore decided to record the response latency from one group of rats in one session and the other in the next session which shared the same environmental stimulus. The animal from the group whose response latency factor was to be measured was put into its chamber first and the timer started immediately afterwards. The second rat was then put into the other chamber and its response latency characteristic ignored. This has been expressed graphically as the mean of two successive sessions of the same environmental treatment. During test sessions only the time-appropriate FRF characteristic and the response rate were considered and have been expressed as the mean value for each experimental session.

6.4. Results

6.4.1. Acquisition of the light/dark discriminative stimulus

The high rate of acquisition of the light/dark cue can be seen in Figs. 6.2 and 6.3, the mean number of sessions to reach criterion being 22.5 (+/- 6.1 s.e.m.). However, there followed soon after a period of below par performance when not enough animals remained at criterion for experiments to be conducted. This raised the mean number of sessions to criterion to 31.25 (+/- 8.3 s.e.m.).

6.4.2. Effect of light and dark training sessions on performance

Response rates during both light and dark periods showed a gradual increase as training progressed with initially a higher rate apparent in the dark phase of the LD cycle. This is depicted in Fig. 6.4 which also shows, in the form of the FRF value, that the quality of responding was similar regardless of the time of the training session and increased as training progressed. Similarly, the response latency parameter, portrayed in Fig. 6.5, was not found to be affected by the lighting/temporal conditions during training.

6.4.3. Effect of melatonin on time-of-day appropriate responding

Melatonin was not found to affect the response rate or the accuracy of responding as measured by the proportion of rats choosing the time-appropriate lever at any of the four time points over the LD cycle investigated (see Figs. 6.6 and 6.7, respectively). The "confidence" characteristic, the time-appropriate FRF value also did not reveal any drug-induced change (see Fig. 6.8). However, the accuracy of animals responding at the EOL stage experiment, which finished before the lights actually went out, was found to be significantly reduced compared to the accuracy of the

animals during experiments conducted at the other four time points (generalized linear regression with binomial error; chi-squared= 24.05, DF=3).

6.4.4. Effect of melatonin on time-of-day appropriate responding with conflicting environmental cue.

Once again, melatonin was found to be without significant effect on the rate, accuracy and confidence of responding in either those rats given the change in lighting in the experimental chamber or those that were pretreated with the appropriate lighting condition (see Figs. 6.9, 6.10, 6.11, respectively). The time-of-day factor was found to be significantly different with respect to the response rate parameter between experiments conducted in light environmental conditions at MD in comparison to those conducted at ML under darkness (one-way ANOVA; $p<0.01$, $F=12.3$, $DF=1, 34$). Pre-treatment with the appropriate lighting condition for 30 min equilibrated the response rates (see Fig. 6.9). The proportion of animals selecting the time-appropriate treatment at MD and ML approached 100%. Reversing the lighting conditions in the experimental chamber had the effect of reducing the proportion of animals choosing the time-appropriate lever regardless of the time-of-day. Although this proportion increased further when animals were previously exposed to 30 min of light or dark (according to whether the experiment was conducted at MD or ML respectively) at least two animals of the seven in each treatment group selected the time-appropriate lever, as shown by Fig. 6.10.

Fig. 6.2. Training curve to light-dark discrimination. (n=7-8).

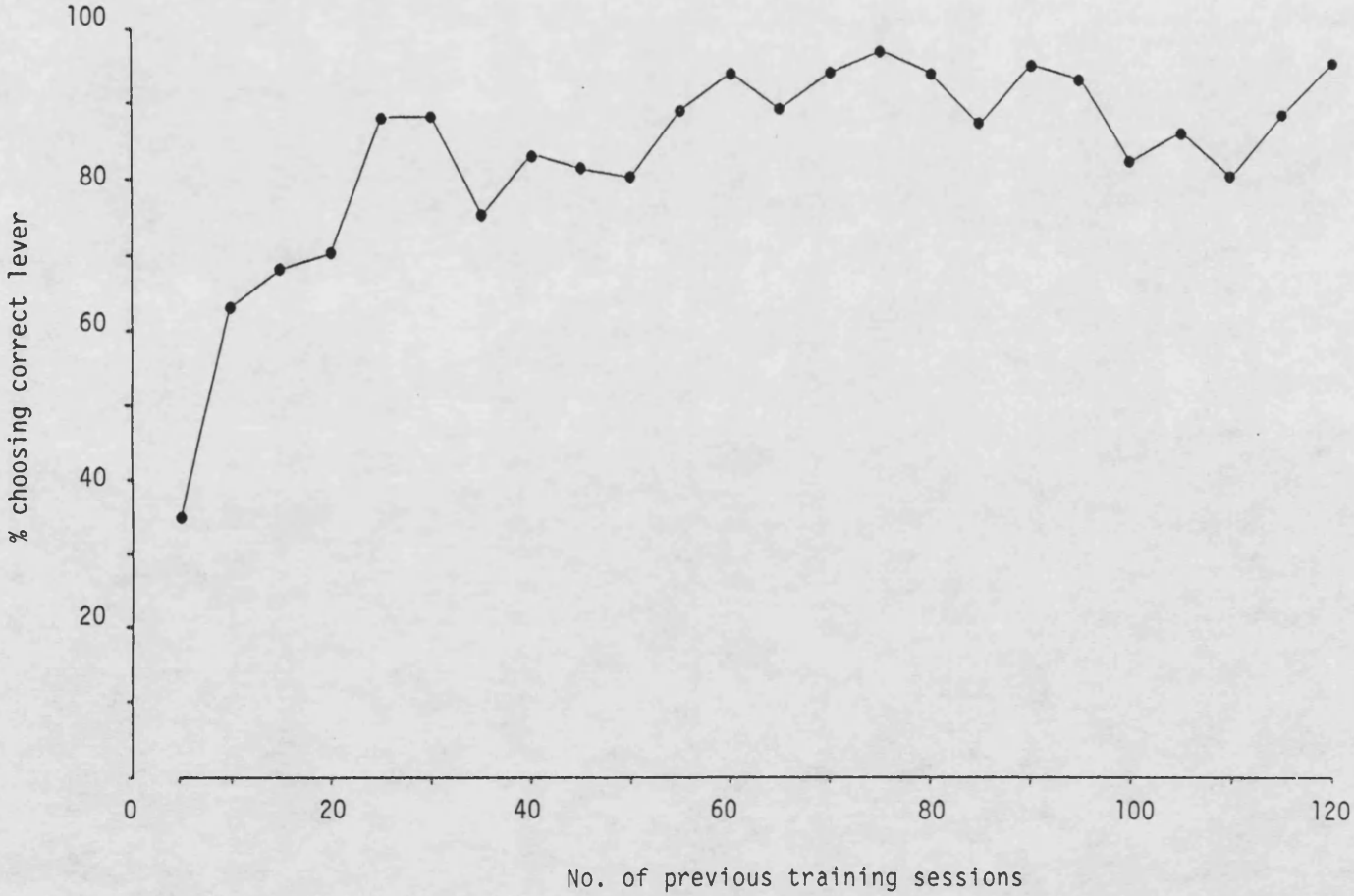


Fig. 6.3. Acquisition of discrimination between light and dark. (n=7-8).

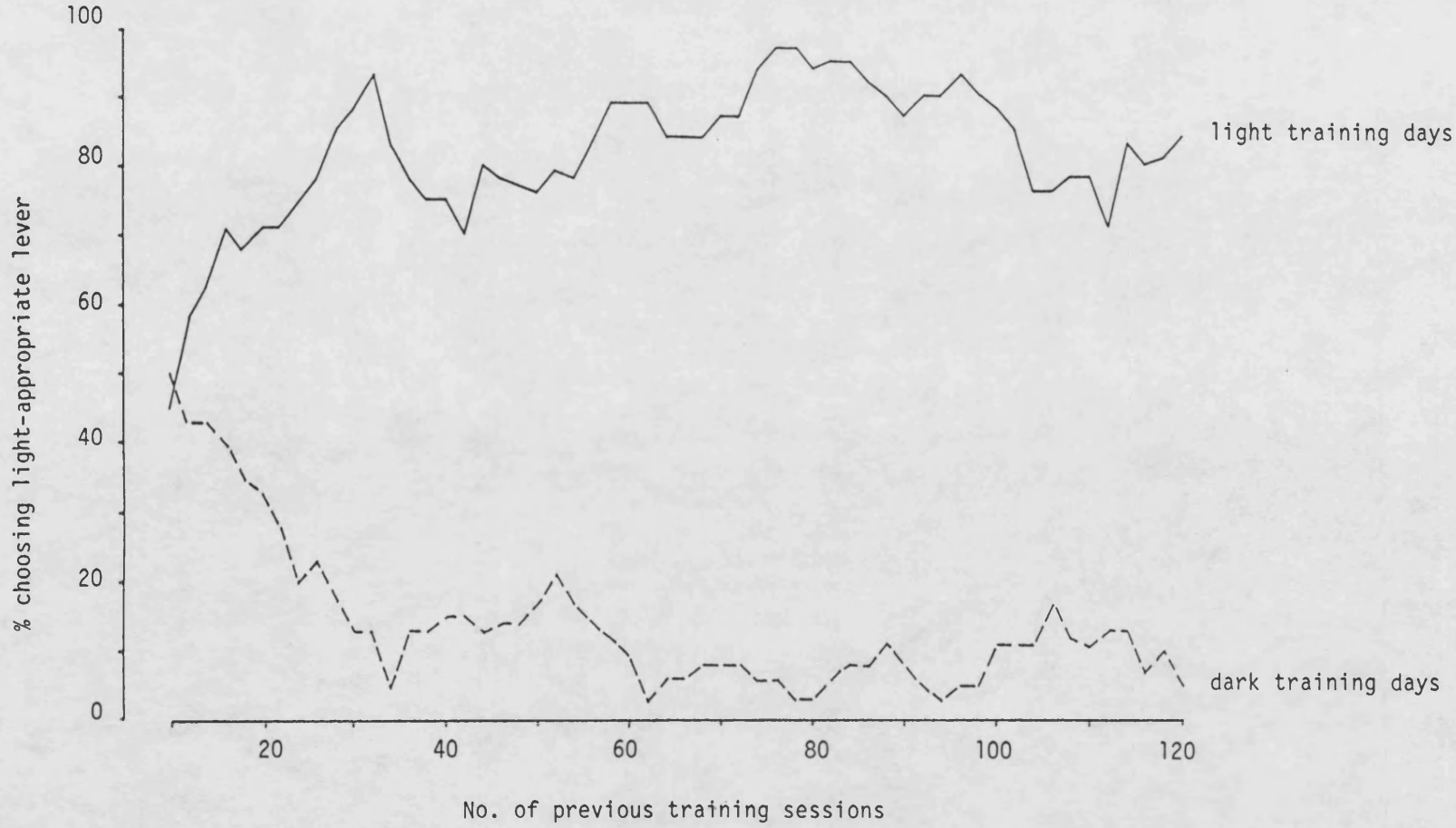


Fig. 6.4. Comparison of FRF values between light and dark training days and comparison of response rate parameter between light and dark training days. Open circles = FRF value light training days, closed circles = FRF value dark training days; open squares = response rate parameter light training days, closed squares = response rate parameters dark training days. (n=7-8).

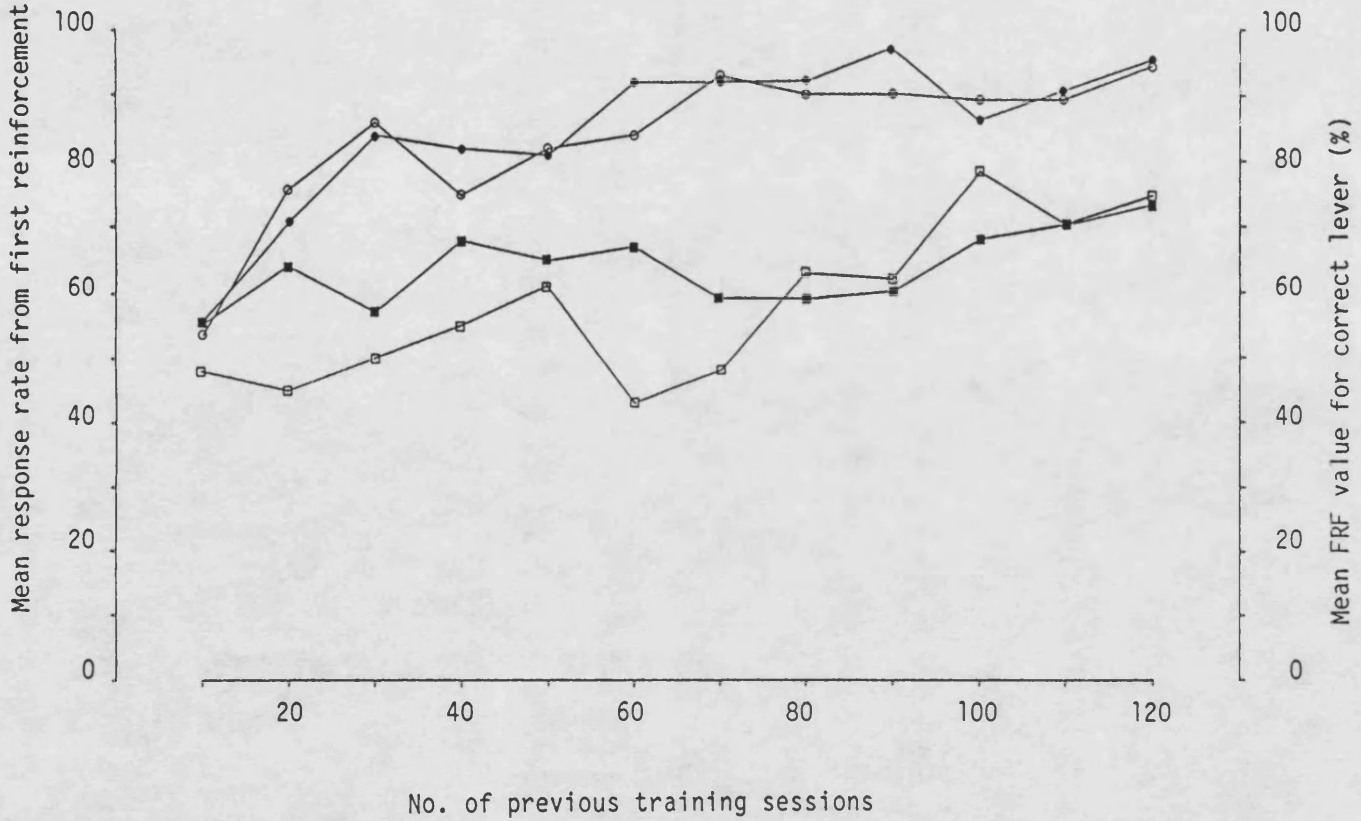


Fig. 6.5. Comparison of response latency times between light and ~~day~~ dark training sessions. (Values represent means of 6-8 rats).

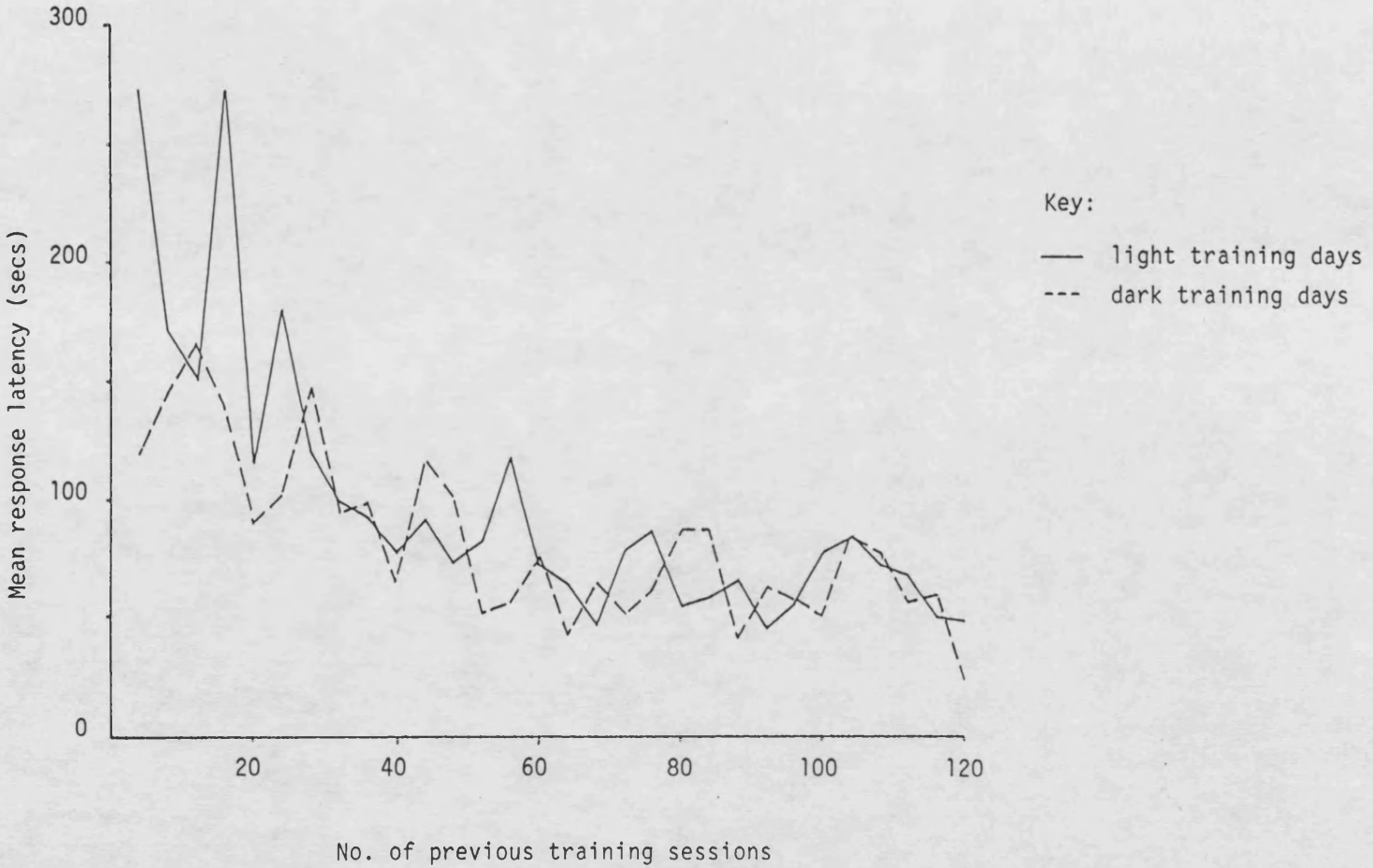
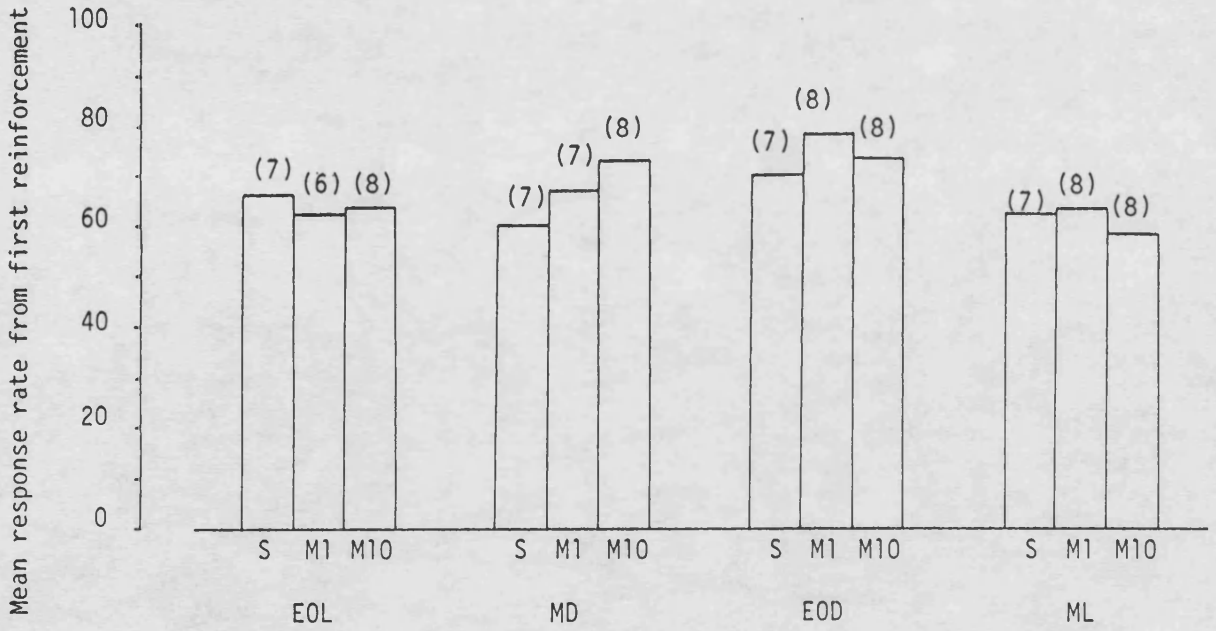


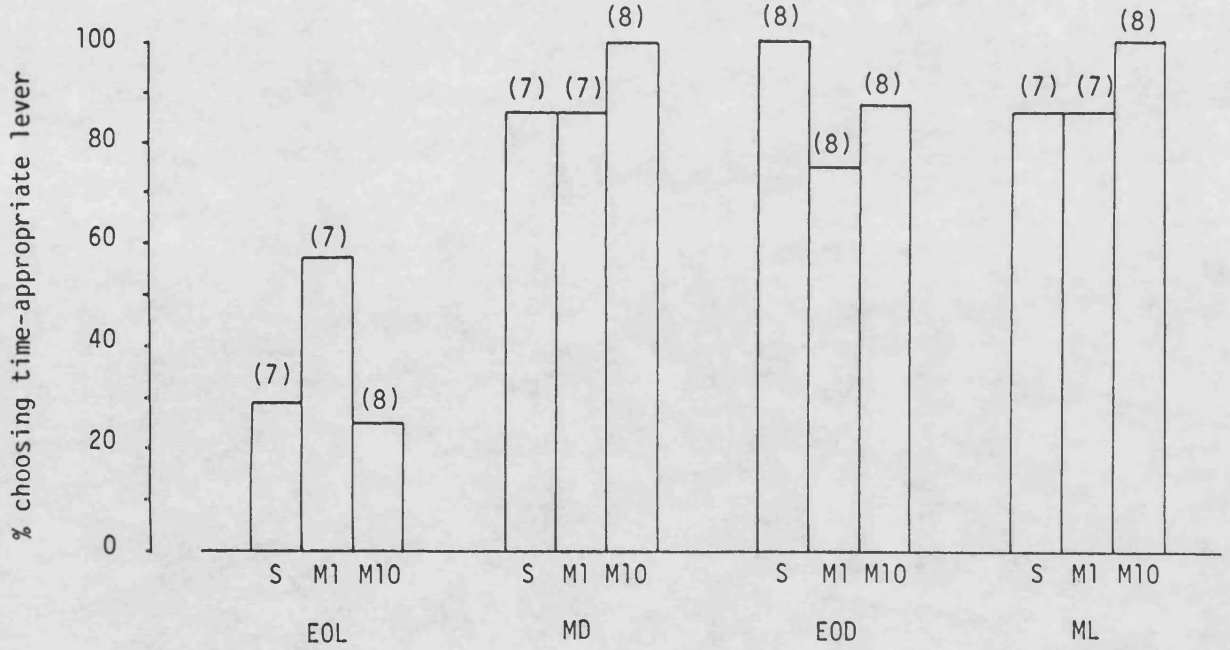
Fig. 6.6. Effect of melatonin on mean response rates (lever press/min) for experiments conducted over four time points of the LD cycle (n=6-8).



Key:

S = Saline
M1 = Melatonin 1mg/kg
M10 = Melatonin 10mg/kg
EOL = End-of-light
EOD = End-of-dark
ML = Mid-light
MD = Mid-dark

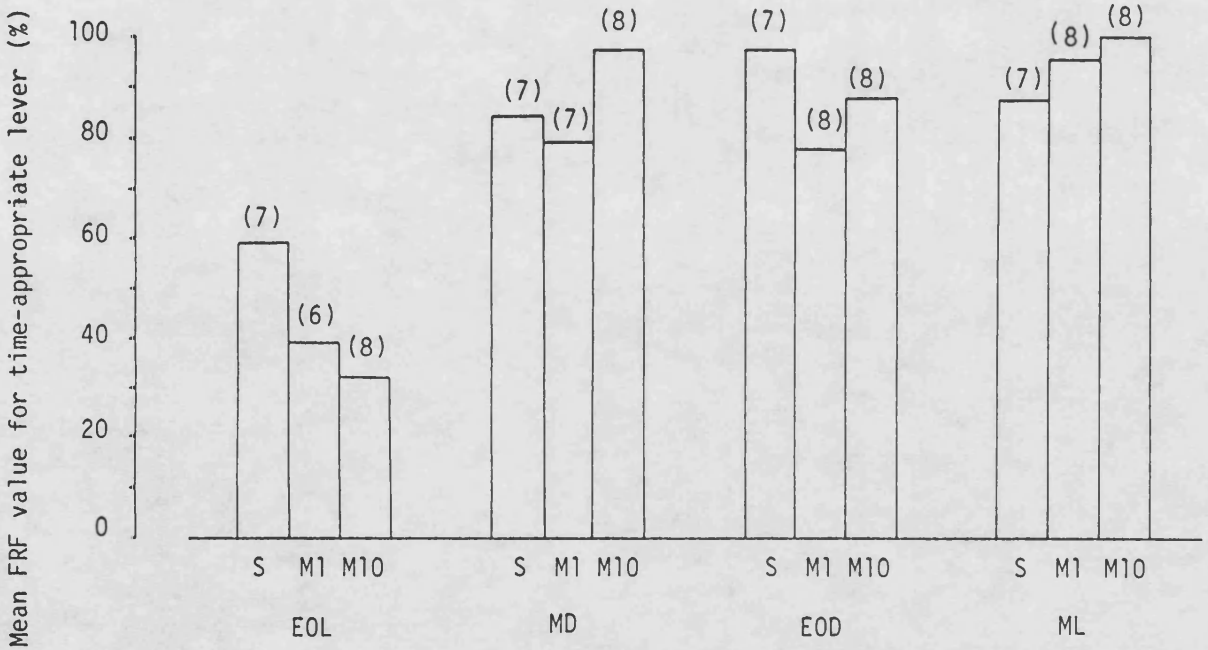
Fig. 6.7. Effect of melatonin on lever choice for experiments conducted at four time points of the LD cycle. (n=7-8).



Key:

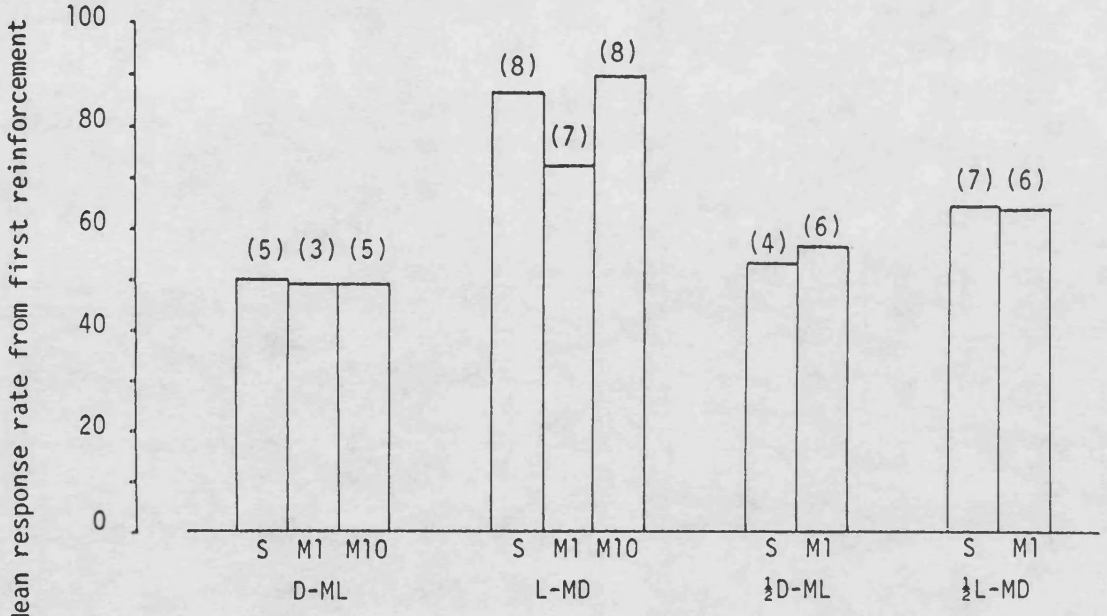
As for Fig. 6.6.

Fig. 6.8. Effect of melatonin on the FRF value for experiments conducted over four time points of the LD cycle. (n=6-8).



Key:
As for Fig. 6.6.

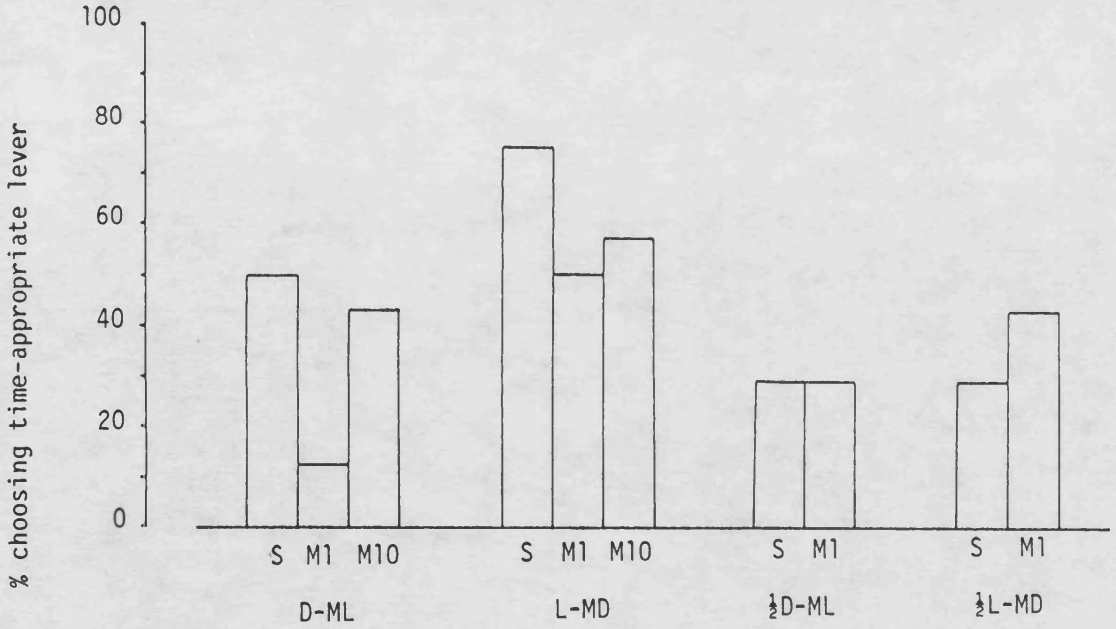
Fig. 6.9. Effect of melatonin on mean response rates (lever presses/min) for experiments using a conflict of visual and temporal discriminative cues. (n=3-8).



Key:

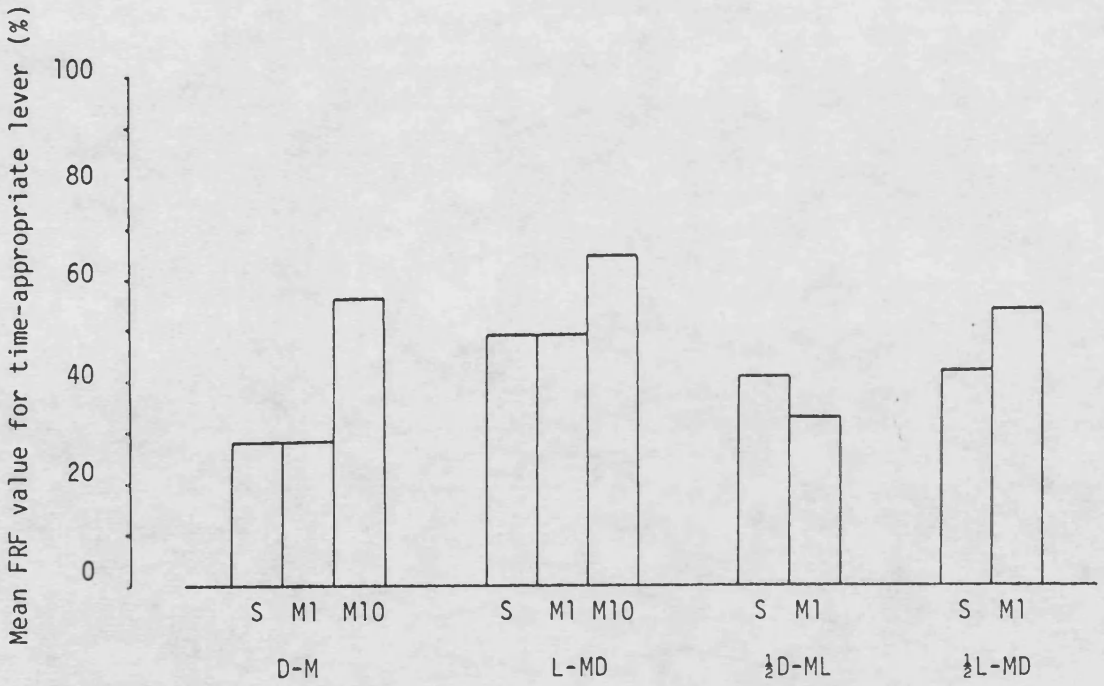
S = Saline
M1 = Melatonin 1mg/kg
M10 = Melatonin 10mg/kg
D-ML = Dark at mid-light (ML)
L-MD = Light at mid-dark (MD)
½D-ML = 30 mins dark treatment at ML
½L-MD = 30 mins light treatment at MD

Fig. 6.10. Effect of melatonin on lever choice for experiments using a conflict of visual and temporal discriminative cues.



Key:
As for Fig. 6.9.

Fig. 6.11. Effect of melatonin on the FRF value for experiments using a conflict of visual and temporal discriminative cues.



Key:

As for Fig. 6.9

6.5. Discussion

This series of experiments were prompted by the thought that although melatonin might alter the cognitive state of a rat to that more appropriate to the dark phase of the LD cycle, this was not a sufficient aberration of normality to be recognized in the form of a discriminative cue by the animal. Since melatonin failed to increase the proportion of subjects pressing the night-appropriate lever in any of the experiments it can be concluded from this work that melatonin does not act as a darkness-related stimulus, at least using this model in the nocturnally active rat. However, certain properties of discriminative behaviour have been revealed. The animals were trained essentially to discriminate a temporal/visual complex cue in a manner analogous to that of the tactile/visual complex cue of Koek and Slangen (1984). When the two components of the discriminative stimulus are presented in conflict with one another to individual animals it is clear that one component takes precedence over the other. If the two stimuli are of equivalent discriminative strength then during conflict experimental sessions one might expect an even split between the two components according to which was perceived as dominant at the time. This would appear to be the case in the experiments described above (see Fig. 6.10) moreover, most animals shifted their responding between the visual and the temporal stimuli as the study progressed. Neither stimulus assumed complete dominance in an animal such that it responded purely according to the prevailing temporal or environmental conditions in every experiment. An alternative explanation for this dichotomous responding is that the cues given in conflict with one another may simply disrupt normal behaviour such that random responding ensues (Koek and Slangen, 1984; Jarbe

and Swedberg, 1982).

The problem in this instance is whether or not the subjects have been trained to discriminate the night/activity-time of the 24 h cycle from the day/rest-time of the cycle. That a significant proportion of the group responded according to the time-of-day rather than the environmental cue when the two component stimuli were given in conflict indicates that temporal parameters were being distinguished from the visual stimulus and that the subjects were not simply reacting to the presence or absence of light. Additionally, the temporal cue proved remarkably resilient in certain individual animals when the group were subjected to 30 min of the visual stimuli before the experimental session. (In future experiments it would be worthwhile to see whether a dose-response curve to this factor could be constructed). It seems likely, therefore, that the animals were trained to a temporal component of the compound stimuli. Less clear is whether that cue corresponded to the nocturnal state of the animal rather than some internal cognitive time-keeper analogous to that responsible for measuring time-dependent schedules in more complex operant conditioning procedures and, ultimately, whether or not this aspect of the cue is actually separate from the nocturnal state of the subject. Within the framework of time measurement might lie the route for an animal to be trained to discriminate between melatonin and saline.

Circadian rhythms and photoperiodism can be viewed as forms of vegetative time-measurement (see Herbert, 1989), that is the internal analysis of time lies beneath the consciousness of the animal which under the influence of external environmental stimuli can be very accurate. The other means of measuring time in the brain is termed cognitive time-measurement which is concerned with the

internal perception of the passage of time and has an accuracy more vulnurable to variability between individual subjects. It would seem probable that cognitive time measurement has the ability to exploit information derived from the vegetative clock (Herbert, 1989). Melatonin is concerned with vegetative time measurement - as demonstrated by its effects on photoperiodism and circadian rhythms. It is not yet clear whether the duration of the night-time melatonin signal or its presence in the plasma at a critical concentration coincident with a sensitive period of target organs is responsible for its action (see Reiter, 1987). (It might well turn out to be a combination of both hypotheses). As mentioned above, a discriminative cue to melatonin might not actually be derived from the direct effect of melatonin on cognition but rather its effect on vegetative time-measurement in communication with cognitive time-measurement. Melatonin was given in all experiments as a pulse, rather than a sustained infusion. This was because melatonin entrained free-running locomotor activity rhythms when the time of injection coincided with the onset of activity (Redman et al., 1983; chapter 5, this thesis). It has been presumed that the melatonin pulse acting on some sensitive region of the brain, probably the SCN, is responsible for eliciting entrainment (Cassone et al., 1986b). The fact that endogenous melatonin subsequently reaches high plasma levels (in comparison to day-time levels) may have been ignored as a factor in this entrainment. This question has been indirectly addressed by Chesworth et al. (1987) who subjected rodents to LL which subsequently demonstrated free-running or disrupted wheel-running and drinking circadian rhythms. Daily melatonin injections led to partial synchronization but not entrainment of free-running rhythms. Chesworth et al. used light

250 and 300 lux which should be sufficient to suppress partially the subjective night-time levels of melatonin in nocturnally-active rats, although no biochemical details were given in the paper. The question raised, therefore is whether sustained release of melatonin into the plasma over 8-12 h would elicit a greater degree of synchronization and perhaps entrainment? This, in turn, leads to the questions concerning the ability of melatonin to act as a discriminative cue. Would animals subjected to LL, sufficient to suppress melatonin secretion, be able to differentiate injections of melatonin from saline? Would animals maintained in DD be able to differentiate melatonin from saline? The complication that regular training sessions might elicit entrainment could be overcome if training times and food availability were randomized over the activity and rest periods of the subjects (if a shock escape discrimination paradigm was used food could be made available *ad libitum*). This could be more easily achieved if locomotor activity was monitored. An alternative strategy, which is more dependent on the vegetative time-measurement mechanism, might be to administer either melatonin or saline as a 12 h s.c. infusion and subject the animals, maintained in LL, to randomized training sessions.

A further consideration is that exteroceptive and interoceptive cues might elicit discrimination via different mechanisms. A psychoactive drug acts upon a particular neurotransmitter system, at least initially, ultimately to elicit a perceptual change in the animal's internal awareness. This may take the form of drowsiness which can make hypnotic drugs like the barbiturates and the benzodiazepines difficult for the animal to differentiate. Alternatively, the cue can be specific for a particular neurotransmitter system, or even a receptor. Thus, an

agent specific for the serotonergic system, that acts as a discriminative stimulus, generates an internal state perceptually different to that generated by an agent specific for, say, the cholinergic system. This can be shown by training animals to discriminate between two drugs (i.e. a drug vs. drug study, rather than drug vs. saline) or using a cholinergic drug as a test compound having first trained a group of rats to discriminate between a serotonergic compound and saline. Distinguishing between cues generated by the activation (or inhibition) of a particular receptor requires the use of antagonism studies and agents with a high specificity of action but such studies can enable the drug discrimination technique to make a significant contribution to receptor subtype classification (see Stolerman et al., 1987). Unless a compound actually disrupts the process of responding, Overton (1966) suggests that an animal trained using a compound specific for one neurotransmitter system or receptor is most likely to reply with saline-appropriate responding if tested with a drug specific for another system or receptor. The same author also propounds that totally random responding during test sessions might reflect a test state that resembles neither training state (Overton, 1966). Disrupted behaviour is usually reflected by non-responding or occasionally by intermediate results (Jarbe and Swedberg, 1982). The discrimination of non-aversive exteroceptive stimuli does not depend upon the generation of any disruptive internal signal. In this instance it can be quite specific; e.g. different types of tactile, visual and sound cues can be distinguished (Jarbe and Johansson, 1984; Koek and Slangen, 1984). In order for discrimination to take place there must be some kind of internal process of interpretation to select the correct response. This process must be based on two

guiding aspects of behaviour - perception and memory - a cue must be perceived and recognized. It may be that distinction between exteroceptive stimuli depends on the activation of highly specific systems of the brain whereas drugs, unless deliberately targetted, elicit generalized effects that disrupt lower levels of brain function. The work of Koek and Slangen (1984) does not suggest that either memory or perception are vulnerable to certain psychoactive drugs, but rather the capacity to respond is affected. Disruption occurred only at the higher doses used and was reflected in a reduced number of animals responding and a reduced degree of responding. It has not been reported that the presence of non-aversive exteroceptive discriminative stimuli disrupt drug-generated cues. Thus, from this argument, since melatonin has no behaviourally-disruptive actions, it would seem unlikely to be capable of affecting the temporal/visual stimulus unless it acted upon the higher centres of the brain responsible for perception and information retrieval. Such activity has not been reliably attributed to melatonin.

The final aspect of this work that should be discussed is the reduction in the number of correct responses, according to time and visual cues, at the EOL phase (see Fig. 6.7). This might reflect a property of anticipatory behaviour. It might also indicate that the onset of the dark period exerts greater influence to overt behaviour than the onset of the light period with respect to nocturnally-active animals since no such disruption of behaviour occurred at the end of the dark phase. Regulated access to food acts as a potent zeitgeber for activity which is resistent to SCN lesioning (Stephan, 1984, 1989). Anticipatory activity is often measured in experiments analysing the effects of feeding and

periodic feeding times on circadian rhythms (e.g. Stephan and Becker, 1989). The nature of the experiments described above meant that access to food was randomized over the light-dark period, and would therefore not act as a zeitgeber. Additionally, animals were maintained on a 12:12 LD cycle. Ideally, simultaneous recording of locomotor activity throughout the course of this experiment would have provided information about the nature of the circadian rhythm of locomotor activity of animals maintained under these circumstances. During the course of the light-dark training programme animals were subjected to several training episodes that took place within 2 h from lights-out, this approximates to one every two weeks. Any erroneous responding during training would, therefore, be incorporated in the results of 5 successive sessions and not show up. Examination of the raw data does not reveal any discrepancy in the accuracy of training sessions conducted within 2 h of lights-out in comparison to those conducted at any other time-point. Another consideration is that experimental sessions took slightly less time to complete than training sessions since animals occupied the operant conditioning chamber for 5 rather than 15 min during experiments. This allowed end-of-light phase experiments to be conducted slightly later than a comparative training episode and hence the last animals subjected to experimentation would be closer to the time of lights-out than those that preceded them. The expected implication, however, namely that the last animals would be more likely to respond erroneously than the others is once again not supported by the raw data. The experiments were repeated at the four time points and the initial results verified.

In conclusion, these time-of-day results suggest that the influence of the pacemaker responsible for anticipatory behaviour

can overcome the discrimination of visual and temporal cues generated by successive training sessions and is not vulnerable to modification by melatonin. It would be of interest to conduct an extended study with larger animal groups and several psychoactive drugs.

CHAPTER 7.

GENERAL DISCUSSION

7. GENERAL DISCUSSION

The results presented in this thesis have already been extensively discussed in each of the relevant chapters. It is the purpose of this final chapter to focus attention upon general aspects of the methods used and to suggest areas where further investigation might be particularly beneficial.

7.1. 5-Hydroxytryptamine

The serotonergic system has been shown to be intimately involved in the expression of a range of unconditioned behaviours. A selection of these, namely locomotor activity, feeding, headtwitches and temperature (though this is not, strictly speaking, a "behaviour"), were used to investigate a possible interaction between the serotonergic system and melatonin as described in chapter 3. Much of this work is based on the argument that the modifications effected by the serotonergic system on these behaviours are mediated by the activation of specific 5-HT receptor subtypes. Evidence to support (and refute) this contention is presented in chapter 3. To be considered here is the fact that these behaviours result from an abnormally active serotonergic system. Thus any melatonin-5-HT interaction would be apparent on a pharmacological basis only and not necessarily have any physiological relevance. The use of non-selective serotonergic antagonists (methysergide and cyproheptadine) by Gaffori and Van Ree (1985) in their investigation of "melatonin-induced behaviour" warrant a repeat study using the more selective agents that have since become widely available before any definitive conclusions (that would account for the origin of the behaviour) can be made.

It would be of interest to use other models of 5-HT_2 receptor activation to test for interactions between the serotonergic system and melatonin. As mentioned in chapter 3, section 3.4, Dugovic (1988, 1989b) has demonstrated the involvement of the 5-HT_2 receptor in melatonin-induced changes in slow-wave sleep. The drug discrimination technique using a 5-HT_2-receptor mediated discriminative stimulus, e.g. that elicited upon the administration of the hallucinogen DOI, is an obvious model to use since it would also complement the work using L-5HTP as the generator of a 5-HT_1-receptor mediated cue.

Observer bias is a necessary factor that must be taken into account with regard to the conclusions reached for the experiments described in this thesis. It was not possible to conduct any study "blind", mainly because drug solutions were prepared immediately before use and certain injections and experiments had to be given during unsocial hours. This in itself introduces the problem of 24 h variability in observer performance. Moreover, the poor solubility of melatonin permits easy identification of control and drug preparations. The headtwitch experiments are most vulnerable to empirical interpretation but the results concerning their circadian rhythm is in agreement with earlier studies in that the maximum number of headtwitches occur during the light phase.

7.2. Drug Discrimination

The subject of bias in drug discrimination has received little formal attention since it was tackled by Rosenthal and Fode (1963). Their extensive study found that the expectations of the experimenter could affect the performance of an animal in learning a T-maze discrimination task. Precisely how this might be achieved is

not known but the authors suggested that information could be conveyed to the animal by, for instance, differences in handling procedures or changes in skin temperature. This seems unlikely in light of the results in chapter 4 which demonstrate that melatonin is not capable of eliciting a discriminative cue perceptible to the animal using the T-maze and Skinner box drug discrimination paradigms, unless, of course, melatonin generated a weak stimulus that could be overcome by signals from the experimenter. It was plausible that discrimination between saline and high dose melatonin/tragacanth could have occurred on the basis of an animal being capable of perceiving the change in viscosity of the injected material from mild suspension (tragacanth/saline) to heavy suspension (melatonin 50mg/kg in tragacanth/saline), but this did not occur.

The method adopted for training animals to learn a discriminative task using the T-maze paradigm inherently minimizes the potential for an animal to discriminate between training and experimental sessions in comparison to the Skinner box paradigm. In training, animals are put into the T-maze one after the other, being returned to the home cage after each trial and then used again for the second series of trials until each animal has received the desired number of trials per training session. The experimental procedure follows the same pattern but each animal receives only one trial. Thus animals cannot perceive a difference between a training and a test session until the first trial has finished. The Skinner box discrimination paradigm requires animals to be put into the operant conditioning chamber for, what is in essence, a single training trial that usually lasts for 10-15 min They are therefore absent from the home cage for a significant period of time in

comparison to an experimental session which can last for between 3-5 min. Experimental sessions are invariably shorter than training sessions to reduce the risk of an animal learning an inappropriate response to a task during an experiment - this is analogous to the single trial given to animals during experiments conducted with the T-maze paradigm. It is possible that animals in the home cage can learn to discriminate between experimental and training sessions according to the duration of absence of one of its conspecifics. Hence the use of an additional "neutral" cage to house animals for the remaining 10 min after a 5 min test session during experiments involving the Skinner box.

Non-uniformity of methodology and discrimination indices between research groups is a problem in drug discrimination experiments (see Colpaert et al., 1976; Colpaert, 1977; Stolerman and D'Mello, 1981). Aside from the differences between equipment and reinforcement/punishment parameters, the schedules to which a group of animals are trained can vary enormously. The experiments conducted for this thesis used a simple FR procedure, but some workers prefer more elaborate schedules which combine two or more conditioning strategies, for example a variable interval (VI) with a FR schedule. This variability in technique is caused by differences of opinion as to which method is best for reducing the probability of animals being able to differentiate between training and experimental sessions, especially if the experimental session adopts an extinction procedure. The extinction procedure involves recording the number of responses on either lever but not providing a reward once the criterion of the schedule has been attained. If the session were allowed to proceed to its natural conclusion less responses would be made until responding ceased altogether. Similarly, at the

other extreme overlong experimental sessions which provide reinforcement once a chioce has been made also carry the risk of disturbing the integrity of the learnt behaviour. Both extremes must be avoided and so experimental sessions of usually between 3 and 5 min are used. Once the test session has finished one of two measurements of "discriminability" can be used - the quantal index or the quantitative index (see Colpaert, 1977; Stolerman and D'Mello, 1981). The former, used for the work described in this thesis, is usually based on the percentage of rats that respond on the drug-appropriate lever at the start of the test session. The quantitative index has most often been calculated as the mean percentage of the total number of responses made upon the drug-appropriate lever during brief test sessions in which no reinforcers are given. Contrary to the earlier work of Colpaert (e.g. Colpaert, 1977) which expresses a preference for the quantal index, Stolerman and D'Mello (1981) have shown that there is a very high correlation between these two parameters and that either can be used as a measure of the discriminative strength of a compound. Due to this range of techniques employed in drug discrimination studies, and most especially if the Skinner box paradigm is used, the most simple procedure was adopted for the purpose of the work carried out for this thesis. There remains, however, a range of schedules that can be used to investigate the general effect of melatonin on operant conditioning behaviour. Of particular interest is the differential reinforcement of low rate responding schedule (DRL). This schedule depends on the ability of an animal to learn an association between time and a pre-determined criterion of responses required for reinforcement. Thus a schedule of DRL-72' indicates that there must be at least 72 seconds between two responses for a

reinforcement to be given, i.e. if an animal presses a lever once at time=0 it is rewarded 72 seconds later provided a second lever-press is not made. Any response within that time resets the clock, and within the time limits of the whole trial, reduces the number of reinforcements that a subject can achieve. This schedule has been used in rats by O'Donnell and Seiden, (1982, 1983) who demonstrated that certain antidepressant drugs (tricyclic antidepressants, atypical antidepressants and MAOIs) increased the number of reinforcements obtained and decreased the total number of responses (i.e. lever-presses). Antipsychotics decreased both the number of reinforcements and the number of responses, suggesting that this heightened temporal awareness was selective for antidepressant treatment. The same group later reported that electroconvulsive therapy had a similar "antidepressant" effect (Seiden et al., 1985). More recent work has also implicated opposing roles for 5-HT_{1A} and 5-HT_2 receptors, in that the 5-HT_{1A} agonist 8-OHDPAT (but not the mixed 5-HT_{1B} and 5-HT_{1C} agonists, mCPP and TFMPP) and the 5-HT_2 antagonists, ketanserin and ritanserin, elicit a similar "anti-depressant-like" effect (Marek et al., 1989a, 1989b). The main question mark over this work is how applicable it is to the clinical situation since it involves analysing acute effects of drugs given repeatedly to the same group of animals, while perhaps the most classical feature of antidepressant therapy is that it can take between 2-4 weeks for a clinical response to become apparent after treatment has begun (Oswald et al., 1972). The relationship between melatonin and depression has already been briefly discussed in chapter 3, section 3.4. The possible role of melatonin and seasonal depression (Seasonal Affective Disorder, or SAD) has received much attention since its description by Lewy et al (1982) who also

reported its successful treatment with bright, white light. Subsequent research has forced a rethink on the role of melatonin in this condition, if it is involved at all (see Thompson, 1988 and references cited therein) although Thompson has speculated upon the involvement of the serotonergic system. Despite this, it would be interesting to investigate the acute and chronic effects of melatonin on rats trained to a DRL conditioning strategy and housed under various lighting schedules, i.e. long photoperiod, short photoperiod and DD and LL. It would also provide a further opportunity to investigate possible interactions between melatonin and the serotonergic system.

The work described in chapter 6 takes to a logical conclusion the concept that melatonin might at as a chemical "dark" signal (see Arendt, 1988). It also examined the effects of melatonin on temporal discrimination in the rat, though not to the precise accuracy required by a DRL schedule of operant conditioning. Melatonin was not found to mimick the cue elicited by the exteroceptive stimulus of darkness in rats. However, if the presence of melatonin generated a weak stimulus this might not have been potent enough for detection. An alternative training technique that is worth investigating would be to pair the supposed melatonin signal with the exteroceptive stimulus in a manner similar to the "overshadowing" procedure adopted by Jarbe and Johansson (1984). Thus melatonin would be administered in conjunction with a dark training session, while saline would be given as a pretreatment with a light training session. Once trained any contribution to the dark stimulus made by melatonin can be tested by substituting a melatonin injection for saline. This also yields further opportunities to study exteroceptive and interoceptive cues in conflict. Caution must

be exercised with this technique, though, since any psychoactive drug capable of generating an interoceptive discriminative stimulus, e.g. pentobarbital, if paired with an exteroceptive stimulus, would form a drug-exteroceptive compound stimulus - a position that might well be expected if melatonin was used as one of the training stimuli (Jarbe and Johansson, 1984; Stolerman et al., 1987). The difference in this instance, however, is that melatonin, unlike the psychoactive drug, on its own, does not elicit a discriminative cue.

7.3. Melatonin

The work described in this thesis relies entirely on behavioural parameters and makes the assumption that they originate from a central source. The work therefore also assumes that melatonin can readily penetrate the brain. The use of an intraperitoneal route of administration for melatonin was chosen on the basis that this was a site where systemic absorption would be rapid via the mesenteric circulation, although there is a significant risk that much of the dose would be lost via hepatic first pass metabolism (Pardridge and Mietus, 1981). However, Sugden (1980) had reported that peak plasma levels of 45ug/ml were attained following an ip dose of 50mg/kg 10 min after injection. Normal plasma melatonin levels vary in the rat between 6pg/ml (daytime nadir) and 75pg/ml (night-time zenith) (cited from Binkley, 1988). Gibbs and Vriend (1981) have reported that the half-life of melatonin in the rat plasma is approximately 20 min, although in mice it is thought to display a biphasic pharmacokinetic pattern with a half life of approximately 2 min, and a secondary half-life of 35 min (Kopin et al., 1961). Thus, although most of the experiments described in the preceding chapters have reported

negative results, it is unlikely to be due to insufficient melatonin reaching the brain reaching the brain. Moreover, 1mg/kg (i.p.) of melatonin was shown to entrain free-running locomotor activity, in agreement with Redman et al. (1983).

Perhaps the best known property of melatonin, that of its effects on mammalian reproductive organs, is not thought to be the result of a direct action (despite putative cytoplasmic binding sites for (^{3}H)-melatonin having been identified in hamster ovaries and rat uterus by Cohen et al. in 1978). Instead, melatonin is thought to regulate the release of the active principles that in turn regulate reproductive state. The picture is complicated further because melatonin is dependent upon the variability in sensitivity of the target organ (e.g. the SCN) in order to behave in this manner (Reiter, 1987; Armstrong, 1989). An extension of this reasoning would be that melatonin has no direct action on any particular neurotransmitter system, but its indirect influence might extend throughout the body according to the sensitivity of the SCN. In this sense, the pineal gland and its principal product, melatonin, rather than acting as a co-ordinator of rhythmic activity in mammals, would appear to act as a tool of the co-ordinator - the SCN, possibly as its "fine-tuner". This would allow it to be very much more flexible in its actions. It would also make an interaction with a particular system more difficult to identify because, together with evidence to suggest that it is a highly potent molecule (e.g. Cassone et al., 1986a; Reiter, 1983b), a small amount could be sufficient to alter the function or characteristics of a neurotransmitter system. A behavioural approach to detect this change which, considering the diurnal variation in melatonin secretion, would not deviate from normality, may simply not be

sensitive enough. The electrophysiological procedure adopted by Mason and Garrett (1990) may hold more promise although their results suggest that the electrophysiological effects of melatonin on select SCN neurones are not mediated by 5-HT. An alternative behavioural approach to this problem, and indeed, the relationship between melatonin and depression as mentioned above, would be to observe patterns and characteristics of social behaviour in an ethological context. It has recently been shown that acute and chronic treatment with certain antidepressants elicit opposing effects on endogenous patterns of social behaviour in rats. Using doses which had little or no effect on the total number of behavioural elements observed during a social interaction test between a resident rat and an unfamiliar intruder, Mitchell (1989, 1990a, 1990b) found that acute antidepressant treatment decreased aggressive and increased flight behaviour exhibited by the resident animal. Acute treatment with the antipsychotic, haloperidol, or the anxiolytic, diazepam, had similar effects but only at doses which concomitantly reduced the total number of behavioural elements observed. Additionally, haloperidol decreased flight-submit behaviour and diazepam reduced environmentally-directed exploratory behaviour. Conversely, chronic antidepressant treatment, but not haloperidol or diazepam, increased the overt aggressive behaviour of resident rats towards the intruder. Increased aggression leading to an increase in rank position within a social hierarchy was also observed in subdominant rats treated chronically with either mianserin or clomipramine. This technique has the disadvantage of being time-consuming, in addition to its dependence on the expertise of the observer to identify and accurately record behavioural elements - making it a rather subjective method. But, together with

the more quantitative operant conditioning procedure discussed above, it may provide a more sensitive means of analysing behavioural manifestations of acute and chronic melatonin treatment in rodents. The ethological technique has an added advantage of being more amenable to investigating the behavioural effects of melatonin on "jet-lagged" rats in a social context.

7.4. Conclusions

The work presented in this thesis allows several deductions to be made although there are a number of areas that require further investigation before definitive conclusions can be drawn. No evidence has been found to substantiate the initial hypothesis that the serotonergic system mediates the effects of melatonin, indeed, melatonin was found to have no direct behavioural effects in the animal models used, excepting its ability to entrain free-running circadian locomotor activity rhythms. Melatonin does not affect the rate or direction of a 180° phase-shift involving 24 h of dark. The question as to whether melatonin influences the phase-shifting ability of appropriately-timed light-pulses on free-running circadian rhythms remains unanswered but worthy of investigation.

The ability of melatonin to act as a discriminative stimulus was explored in chapters 4 and 6. At the doses employed melatonin did not elicit a discriminative stimulus and has no influence on the discriminative cue generated by L-5HTP. Moreover, melatonin was found to be without effect on both the visual and temporal components of a combined light:dark/time-of-day discriminative cue. Interestingly, the results suggest that in this circumstance of using operant conditioning, anticipatory behaviour can overcome visual and temporal discriminative cues.

Finally, the most intriguing deduction that can be made from this work stems from the fact that melatonin can entrain free-running rhythms but does not elicit a discriminative stimulus. Thus melatonin does not appear to alter the cognitive state of the rat in a manner that is perceptible to the animal. This suggests that entrainment is not dependent on the periodic generation of an internal signal that the animal is "consciously" aware of. It would be of interest to compare the capacity of a range of psychoactive compounds to generate a discriminative stimulus, with their ability to entrain free-running circadian rhythms.

REFERENCES

REFERENCES

Aghajanian, G.K., Foote, W.E. and Sheard, M.H., (1970). Action of psychotogenic drugs on single midbrain raphe neurons. J. Pharmacol. Exp. Ther., 171, 178-187.

Aghajanian, G.K. (1981). Modulatory role of serotonin at multiple receptors in brain. In: Serotonin Neurotransmission and Behaviour. Eds. Jacobs, B.L. and Gelperin, A., pp. 156-185. London: The MIT Press.

Ahlenius, S., Larsson, K., Svensson, L., Hjorth, S., Carlsson, A., Lindberg, P., Wikstrom, H., Sanchez, D., Arvidsson, L.E., Hacksell, U. and Nilsson, J.L.G. (1981). Effects of a new type of 5-HT receptor agonist on male rat sexual behaviour. Pharmacol. Biochem. Behav., 15, 785-792.

Albers, H.E. and Ferris, C.F. (1984). Neuropeptide Y: A possible neurotransmitter in light-dark entrainment of circadian rhythms. Soc. Neurosci. Abstr., 10, 502.

Albrecht, P., Vissher, M.B., Bittner, J.J. and Halberg, F. (1956). Daily changes in 5-hydroxytryptamine concentration in mouse brain. Proc. Soc. Exp. Biol. Med., 92, 703-706.

Aloyo, V.J. and Walker, R.F. (1987). Noradrenergic stimulation of serotonin release from rat pineal glands *in vitro*. J. Endocr., 114, 3-9.

Aloyo, V.J. and Walker, R.F. (1988). Alpha-adrenergic control of serotonin release from rat pineal glands. Neuroendocrinology, 48, 61-66.

Altman, J. (1962). Diurnal activity rhythm of rats with lesions of superior colliculus and visual cortex. Am. J. Physiol., 202, 1205-1207.

Anden, N. E., Dahlstrom, A., Fuxe, K., Larsson, K., Olson, L. and Ungerstedt, U. (1966). Ascending monoamine neurons to the telencephalon and diencephalon. Acta. Physiol. Scand., 67, 313-326.

Anton-Tay, F. and Wurtman, R.J. (1969). Regional uptake of [^{3}H]-melatonin from blood or cerebrospinal fluid in the rat brain. Nature, 221, 474-475.

Anton-Tay, F., Chou, C., Anton, S. and Wurtman, R. (1968). Brain serotonin concentration: Elevation following intraperitoneal administration of melatonin. Science, 162, 277-278.

Anton-Tay, F., Diaz, J.L. and Guardiola-Fernandez, A. (1971). On the effect of melatonin on the human brain. Its possible therapeutic implications. Life Sci., 10, 841-850.

Arendt, J. (1988). Melatonin and the human circadian system. In: Melatonin: Clinical Perspectives. Eds. Miles, A., Philbrick, D.R.S. and Thompson C., pp. 43-61. Oxford: Oxford University Press.

Arendt, J. and Marks, V. (1983). Can melatonin alleviate jet-lag? Brit. Med. J., 287, 426.

Arendt, J., Bojkowski, C., Folkard, S., Franey, C., Marks, V., Minors, D., Waterhouse, J., Wever, R.A., Wildgruber, C. and Wright, J. (1985). Some effects of melatonin and the control of its secretion in humans. In: Photoperiodism, melatonin and the pineal. Ciba Foundation Symposium 117., pp. 266-283. London: Pitman.

Arendt, J., Aldhous M. and Marks, V. (1986) Alleviation of jet-lag by melatonin: preliminary results of controlled double-blind trial. Brit. Med. J., 292, 1170.

Arendt, J., Aldhous M., Marks, M., Folkard, S., English, J., Marks, V. and Arendt, J.H. (1987). Some effects of jet-lag and its treatment by melatonin. Ergonomics, 30, 1379-1393.

Armstrong, S.M. (1989). Melatonin and circadian control in mammals. Experientia, 45, 932-938.

Armstrong, S.M. and Redman, J. (1985). Melatonin administration: effects on rodent circadian rhythms. In: Photoperiodism, melatonin and the pineal. Ciba Foundation Symposium 117, pp. 188-207. London: Pitman.

Arutyunyan, G.S., Mashkovskii, M.D. and Roshchina, L.F. (1964). Pharmacolgical properties of melatonin. Fed. Proc., 23, 1330-1332.

Ashcroft, G.W., Eccleston, D. and Crawford, T.B.B. (1965). 5-hydroxyindole metabolism in rat brain. A study of intermediate metabolism using the technique of tryptophan loading. J. Neurochem., 12, 483-492.

Auerbach, D.A., Klein, D.C., Woodard, C. and Auerbach, G.D. (1981). Neonatal rat pinealocytes: typical and atypical characteristics of [^{125}I]-iodohydroxy-benzylpindolol binding and adenosine 3',5'-monophosphate accumulation. Endocrinology, 108, 559-567.

Arvidsson, L.E., Hacksell, U., Nilsson, J.L.G., Hjorth, S., Carlsson, A., Lindberg, P., Sanchez, D. and Wikstrom, H. (1981). 8-Hydroxy-2-di-n-propylamino) tetralin, a new centrally acting 5-hydroxytryptamine receptor agonist. J. Med. Chem., 24, 921-923.

Axelrod, J. and Weisbach, H. (1960). Enzymatic O-methylation of N-acetylserotonin to melatonin. Science, 131, 1312.

Axelrod, J. and Weisbach, H. (1961). Purification and properties of hydroxyindole-O-methyltransferase. J. Biol. Chem., 236, 211-213.

Axelrod, J., Wurtman, R.J. and Snyder, S. (1965). Control of hydroxyindole-O-methyltransferase activity in the rat pineal gland by environmental lighting. J. Biol. Chem., 240, 949-954.

Azmitia, E.C. and Segal, M. (1978). An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei of the rat. J. Comp. Neurol., 179, 641-668.

Banky, Z., Halasz, B. and Nagy, G. (1986). Circadian corticosterone rhythm did not develop in rats seven weeks after destruction of the serotonergic nerve terminals in the suprachiasmatic nucleus at the age of 16 days. Brain Res., 369, 119-124.

Banky, Z., Molnar, J., Csernus, V. and Halasz, B. (1988). Further studies on circadian hormone rhythms after local pharmacological destruction of the serotonergic innervation of the rat suprachiasmatic region before the onset of the corticosterone rhythm. Brain Res., 445, 222-227.

Barbaccia, M.L., Gandolfi, O., Chuang, D.M. and Costa, E. (1983a). Differences in the regulatory adaption of the 5-HT2 recognition sites labelled by [^{3}H]-mianserin and [^{3}H]-ketanserin. Neuropharmacology, 22, 123-124.

Barbaccia, M.L., Brunello, N., Chuang, D.M. and Costa E. (1983b). Serotonin-elicited amplification of adenylate cyclase activity in hippocampal membranes from adult rat. J. Neurochem., 40, 1671-1679.

Barchas, J. and Usdin, E. (Eds.) (1973). Serotonin and Behaviour. New York: Academic Press.

Barchas, J., Da Costa, F. and Spector, S. (1967). Acute pharmacology of melatonin. Nature, 214, 919-920.

Barrett, R.J., Blackshear, M.A., Sanders-Bush, E. (1982). Discriminative stimulus properties of L-5-Hydroxytryptophan: behavioural evidence for multiple serotonin receptors. Psychopharmacology, 76, 29-35.

Barry, H., III. (1968). Prolonged measurements of discrimination between alcohol and nondrug states. J. Comp. Physiol. Psychol., 65, 349-352.

Barry, H., III. (1974). Classification of drugs according to their discriminable effects in rats. Fed. Proc., 33, 1814-1824.

Barry, H., III. and Krimmer, E.C., (1978). Pharmacology of discriminative drug stimuli. In: Drug Discrimination and State Dependent Learning. Eds. Ho, B.T., Richards, D.W. and Chute, D.L., pp. 3-32. New York: Academic. Press.

Barry, H., III., Koepfer, E. and Lutch, J. (1965). Learning to discriminate between alcohol and non-drug condition. Psychol. Reports, 16, 1072.

Baxter, M.G., Miller, A.G. and Sorono, F.E. (1970). The effect of cyproheptadine on food consumption in the fasted rat. Br. J. Pharmacol., 39, 229-230.

Bedard, P. and Pycock, C.J. (1977). "Wet-dog" shake behaviour in the rat: a possible quantitative model of central 5-hydroxytryptamine activity. Neuropharmacology, 16, 663-670.

Bendotti, C. and Samanin, R. (1986). 8-Hydroxy-2-(di-n-propylamino) tetralin (8-OHDPAT) elicits eating in free-feeding rats by acting on

central serotonin neurons. Eur. J. Pharmacol., 121, 147-150.

Bennett, J.L. and Aghajanian, G.K. (1974). D-LSD binding to brain homogenates: possible relationship to serotonin receptors. Life Sci., 15, 1935-1944.

Bennett, J.P. and Snyder, S.H. (1975). Stereospecific binding of d-lysergic acid diethylamide (LSD) to brain membranes: Relationship to serotonin receptors. Brain Res., 94, 523-544.

Bennett, J.P. and Snyder, S.H. (1976). Serotonin and lysergic acid diethylamide binding in rat brain membranes: relationship to postsynaptic serotonin receptors. Mol. Pharmacol., 12, 373-389.

Benus, R.F., Kodhaas, J.M. and Van Oortmersen, G.A. (1988). Aggression and adaption to the light-dark cycle: Role of intrinsic and extrinsic control. Physiol. Behav., 43, 131-137.

Bernardis, L.L. (1973). Disruption of diurnal feeding and weight gain cycles in weanling rats in ventromedial and dorsomedial-hypothalamic lesions. Physiol. Behav., 10, 855-861.

Binkley, S. (1988). The Pineal: Endocrine and Neuroendocrine Function. New Jersey: Prentice Hall.

Binkley, S.A., Kluth, E. and Menaker, M. (1971). Pineal function in sparrows: circadian rhythms and body temperature. Science, 174, 311-314.

Binkley, S., Hryshchyshy, M. and Reilly, K. (1979). N-acetyl-transferase activity responds to environmental lighting in the eye as well as in the pineal gland. Nature, 281, 479-481.

Bittman, E.L. (1984). Melatonin and photoperiodic time measurement: Evidence from rodents and ruminants. In: The Pineal Gland. Ed. Reiter, R.J., pp. 155-192. New York: Raven Press.

Blackmore, P.F., Bocckino, S.B., Waynick, L.E. and Exton, J.H. (1985). Role of a guanine nucleotide binding regulatory protein in the hydrolysis of hepatocyte phosphatidylinositol-4,5-biphosphate by calcium-mobilizing hormones and the control of cell calcium. J. Biol. Chem., 260, 14477-14483.

Bloom, F.E., Hoffer, B.J., Siggins, G.R., Barker, J.L. and Nicoll, R.A. (1972) Effects of serotonin on central neurons: Micro-iontophoretic administration. Fed. Proc. 31, 97-106.

Blundell, J.E. (1977). Is there a role for serotonin (5-hydroxytryptamine) in feeding? Int. J. Ob., 1, 15-42.

Blundell, J.E. and Lesham, M.B. (1975). The effect of 5-hydroxy-tryptophan on food intake and on the anorectic action of amphetamine and fenfluramine. J. Pharm. Pharmacol., 27, 31-37.

Bouhelal, R., Smounya, L. and Bockaert, J. (1988). 5-HT_{1B} receptors are negatively coupled with adenylate cyclase in rat substantia nigra. Eur. J. Pharmacol., 151, 189-196.

Boullin, D.J. (1978). Biochemical indicators of central serotonin function. In: Serotonin in mental abnormalities. Ed. Boullin D.J., pp. 1-28. Chichester: J. Wiley and Sons.

Bradley, P.B., Engel, G., Feniuk, W., Fozard, J.R., Humphrey, P.P.A., Middlemiss, D.N., Mylecharane, E J., Richardson, B.P. and Saxena, P.R. (1986). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology, 25, 563-576.

Broadway, J., Folkard, S. and Arendt, J. (1987). Bright light phase shifts the human melatonin rhythm in Antarctica. Neurosci. Letts., 79, 185-189.

Brown, G.M., Young, S.N., Gauthier, S., Tsui, H. and Grota, L.J. (1979). Melatonin in human cerebrospinal fluid in the daytime: its origin and variation with age. Life Sci., 25, 929-936.

Bubenik, G.A., Brown, G.M., Uhlir, T. and Grota, L.A. (1974). Immunohistochemical localization of N-acetylindolealkylamines in pineal gland, retina and cerebellum. Brain Res., 81, 233-242.

Bubenik, G.A., Purtill, R.A., Brown, G.A. and Grota, L.A. (1978). Melatonin in the retina and the Harderian gland: ontogeny, diurnal variations and melatonin treatment. Experimental Eye Research, 27, 323-334.

Burns, T.G. and Brown, G.M. (1984). The effect of acute and chronic desmethylimipramine treatment on pineal and serum melatonin and N-acetylserotonin. Adv. Biosci., 53, 25-30.

Card, J.P. and Moore, R.Y. (1982). Ventral lateral geniculate nucleus efferents to the rat suprachiasmatic nucleus exhibit avian pancreatic polypeptide-like immunoreactivity. J. Comp. Physiol., 206, 390-396.

Cardinali, D.P. and Rosner, J.M. (1971). Retinal localization of the hydroxyindole-O-methyltransferase (HIOMT) in the rat. Endocrinology, 89, 301-303.

Cardinali, D.P. and Wurtman, R.J. (1972). Hydroxyindole-O-methyl transferases in rat pineal, retina and Harderian gland. Endocrinology, 91, 247-252.

Cardinali, D.P. and Friere, F. (1975). Melatonin effects on brain. Interaction with microtubule protein, inhibition of fast axoplasmic flow and induction of crystalloid and tubular formations in the hypothalamus. Mol. Cell. Endocr., 2, 317-330.

Cardinali, D.P., Nagle, C.A., Friere, F. and Rosner, J.M. (1975). Effects of melatonin on neurotransmitter uptake and release by synaptosome-rich homogenates of the rat hypothalamus. Neuroendocrinology, 18, 72-85.

Cardinali, D.P., Vacas, M.I. and Boyer, E.E. (1979). Specific binding of melatonin in bovine brain. Endocrinology, 105, 437-441.

Cardinali, D.P., Ritta, M.N., Pereyra, E. and Solveyra, C.G. (1982). Role of prostaglandins in rat pineal neuroeffector junction: Changes in melatonin and norepinephrine release *in vitro*. Endocrinology, 111, 530-534.

Carli, M., Invernizzi, R., Cervo, L. and Samanin, R. (1988). Neurochemical and behavioural studies with RU-24969. Psychopharmacology, 94, 359-364.

Carlsson, A. (1974). Measurements of monoamine synthesis and turnover with special reference to 5-hydroxytryptamine. Adv. Biochem. Psychopharmacol., 10, 75-81.

Carter, D.S. and Goldman, B.D. (1983a). Antigonadal effects of timed melatonin infusions in pinealectomized male Djungarian hamsters (*Phodopus sungorus sungorus*): Duration is the critical parameter. Endocrinology, 113, 1261-1267.

Carter, D.S. and Goldman, B.D. (1983b). Progonadal role of the pineal in the Djungarian hamster (*Phodopus sungorus sungorus*): mediation by melatonin. Endocrinology, 113, 1268-1273.

Cassone, V.M., Chesworth, M.J. and Armstrong, S.M. (1986a). Dose-dependent entrainment of rat circadian rhythms by daily injection of melatonin. J. Biol. Rhythms, 1, 219-229.

Cassone, V. M., Chesworth, M.J. and Armstrong, S.M. (1986b). Entrainment of rat circadian rhythms by daily injections of melatonin depend upon the hypothalamic suprachiasmatic nuclei. Physiol. Behav., 36, 1111-1121.

Cawthon, R.M., Pintar, J.E., Haseltine, F.P. and Breakefield, X. O. (1981). Differences in the structure of A and B forms of human monoamine oxidase. J. Neurochem., 37, 363-372.

Chamblin, M.H. (1973). Melatonin: An assessment of a possible role in the induction and maintenance of sleep in the normal and parachorophenylalanine treated cat. Diss. Abstr. Int., B33, 4535.

Chan, A. and Ebadi, M. (1980). The kinetics of NE-induced stimulation of SNAT in bovine pineal gland. Neuroendocrinology, 31, 244-251.

Chase, P.A., Seiden, L.S. and Moore, R.Y. (1969). Behavioural and neuroendocrine responses to light mediated by separate visual pathways in the rat. Physiol. Behav., 4, 949-952.

Chatfield, C. (1985). The analysis of Time-Series. 3rd edition (reprinted). London: Chapman and Hall.

Cheifetz, S. and Warsh, J.J. (1980). Occurrence and distribution of 5-hydroxytryptophol in the rat. J. Neurochem., 34 , 1093-1099.

Chesworth, M.J., Cassone, V.M. and Armstrong, S.M. (1987). Effects of daily melatonin injections on activity rhythms of rats in constant light. Am. J. Physiol., 253, R101-R107.

Cheung, P. and McCormack, P. (1982). Failure of pinealectomy or melatonin to alter circadian rhythm of the rat. Am. J. Physiol., 242, R261-R264.

Chik, C.L., Ho, A.K. and Klein, D.C. (1988a). alpha$_1$-Adrenergic potentiation of vasoactive intestinal peptide stimulation of rat pineal adenosine 3',5'-monophosphate and guanosine 3'5'-monophosphate: Evidence for a role of calcium and protein kinase C. Endocrinology, 122, 702-708.

Chik, C.L., Ho, A.K. and Klein, D.C. (1988b). Dual receptor regulation of cyclic nucleotides: Alpha$_1$-adenergic receptor potentiation of vasoactive intestinal peptide stimulation of pinealocyte adenosine 3'5'-mono-phosphate. Endocrinology, 122, 1646-1651.

Chu, E.W., Wurtman, R.J. and Axelrod, J. (1964). An inhibitory effect of melatonin on the estrous phase of the estrous cycle of the rodent. Endocrinology, 75, 238-242.

Clark, M.L., Dourish, C.T. and Iversen S.D. (1988). Low doses of the 5-HT$_1$ agonist RU 24969 induce sedation in the mouse: Evidence for mediation by 5-HT$_{1B}$ autoreceptors. Br. J. Pharmacol., 95, 499P.

Clarke, J.D. and Coleman, G.J. (1986). Persistent meal-associated rhythms in SCN-lesioned rats. Physiol. Behav., 36(1), 105-113.

Cohen, M., Roselle, D., Chabner, B., Schmidt, T.J. and Lippman, M. (1978). Evidence for a cytoplasmic melatonin receptor. Nature, 274, 894-895.

Collu, R., Fraschini, F. and Martini, L. (1971). Blockade of ovulation by melatonin. Experientia, 27, 844-845.

Colpaert, F.C. (1977). Drug-induced cues and states: Some theoretical and methodological inferences. In: Discriminative Stimulus Properties of Drugs. Ed. Lal, H., pp. 5-21. New York: Plenum Press.

Colpaert, F.C., (1982). Increased naloxone reversibility in fentanyl dose-dose discrimination. Eur. J. Pharmacol., 84, 229-231.

Colpaert, F.C. (1988). Intrinsic activity and discriminative effects of drugs. In: Transduction Mechanisms of Drug Stimuli. Eds. Colpaert, F.C. and Balster, R.L., pp. 154-160. Berlin: Springer-Verlag.

Colpaert, F.C. and Janssen, P.A.J. (1983). A characterization of LSD-antagonist effects of pirenperone in the rat. Neuropharmacology, 22, 1001-1005.

Colpaert, F.C., Niemegeers, C.J.E. and Janssen, P.A.J. (1982). A drug discrimination analysis of lysergic acid diethylamide (LSD): *In vivo* agonist and antagonist effects of purported serotonin antagonists and of pirenperone, an LSD-antagonist. J. Pharmacol. Exp. Ther., 221, 206-214.

Colpaert, F.C., Meert, T.F., Niemegeers, C.J.E. and Janssen, P.A.J. (1985). Behavioural and 5-HT antagonist effects of ritanserin: A pure and selective antagonist of LSD discrimination in rat. Psychopharmacology, 86, 45-54.

Conn, P.J. and Sanders-Bush, E. (1984). Selective 5-HT2 antagonists inhibit serotonin stimulated phosphatidylinositol metabolism in cerebral cortex. Neuropharmacology, 23, 993-996.

Conn, P.J. and Sanders-Bush, E. (1987). Central serotonin receptors: effector systems, physiological roles and regulation. Psychopharmacology, 92, 267-277.

Conn, P.J., Sanders-Bush, E., Hoffman, B.J. and Hartig, P.R. (1986). A unique serotonin receptor in the choroid plexus is linked to phosphatidylinositol turnover. Proc. Natl. Acad. Sci. USA., 83, 4086-4088.

Conn, P.J., Janowsky, A. and Sanders-Bush, E. (1987). Denervation supersensitivity of 5-HT1c receptors in rat choroid plexus. Brain Res., 400, 396-398.

Cooper, J.R., Bloom, F.E. and Roth, R.H. (1986). The Biochemical Basis of Neuropharmacology, 5th edition. New York: Oxford University Press.

Corne, S.J., Pickering, R.W. and Warner, B.T. (1963). A method for assessing the effect of drugs on the central actions of 5-hydroxytryptamine. Br. J. Pharmacol., 20, 106-120.

Cortes, R., Palacios, J.M. and Pazos, A. (1984). Visualization of multiple serotonin receptors in the rat brain by autoradiography. Br. J. Pharmacol., 82, 202P.

Craft, C.M., Morgan, W.W., Jones, D.J. and Reiter, R.J. (1985). Hamster and rat pineal gland beta-receptor characterization with iodocyanopindolol and the effect of decreased catecholamine synthesis on the receptor. J. Pineal Res., 2, 51-66.

Cramer, H., Rudolph, J., Consbruch, U. and Kendel, K. (1974). On the effects of melatonin on sleep and behaviour in man. Adv. Biochem. Psychopharmacol., 11, 187-191.

Cunningham, K.A. and Appel, J.B. (1986). 5-hydroxytryptamine-1 (5-HT1) receptor involvement in the stimulus properties of 1-(m-trifluoromethylphenyl) piperazine (TFMPP). J. Pharmacol. Exp. Ther., 237, 369-377.

Cunningham, K.A. and Appel, J.B. (1987). Neuropharmacological reassessment of the discriminative stimulus proprties of d-Lysergic acid diethylamide (LSD). Psychopharmacology, 91, 67-73.

Cunningham, K.A., Callahan, P.M. and Appel, J.B. (1985). Differentiation between the stimulus effects of 1-5-hydroxy-tryptophan and LSD. Eur. J. Pharmacol., 108, 179-186.

Cunningham, K.A., Callahan, P.M. and Appel, J.B. (1987). Discriminative stimulus properties of 8-hydroxy-2-(di-N-propylamino) tetralin (8-OHDPAT): Implications for understanding the actions of novel anxiolytics. Eur. J. Pharmacol., 138, 29-36.

Czeisler, C.A., Allan, J.S., Strogatz, S.H., Ronda, J.M., Sanchez, R., Rios, C.D., Freitag, W.O., Richards, G.S. and Kronauer, R.E. (1986). Bright light resets the human circadian pacemaker independent of the timing of the sleep-wake cycle. Science, 233, 667-671.

Daan, S. and Pittendrigh, C.S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents. II. The variability of phase response curves. J. Comp. Physiol., 106, 253-266.

Dafny, N. (1980). Photic input to rat pineal gland conveyed by both sympathetic and central afferents. J. Neural. Transm., 48, 203-208.

Dahlstrom, A. and Fuxe, K. (1965). Evidence for the existence of monoamine-containing neurons in the central nervous system. 1. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta. Physiol. Scand., 62 (Suppl. 232), 1-55.

Dark, J. and Asdourian, D. (1975). Entrainment of the rat's activity rhythm by cyclic light following lateral geniculate nucleus lesions. Physiol. Behav., 15, 295-301.

Datta, P.C. and King, M.G. (1980). Melatonin: Effects on brain and behaviour. Neurosci. Biobehav. Rev., 4, 451-458.

Davies, J.A., Navaratnam, V. and Redfern, P.H. (1973). A 24-hour rhythm in passive avoidance behaviour in rats. Psychopharmacologia (Berlin), 32, 211-214.

Deguchi, T. (1977). Tryptophan hydroxylase in pineal gland of rat: Postsynaptic localization and absence of circadian change. J. Neurochem., 28, 667-668.

Deguchi, T. and Barchas, J.D. (1972). Effect of p-chlorophenylalanine on tryptophan hydroxylase in rat pineal. Nature, 235, 92-93.

Demaine, C. and Kann, H.C. (1979). Modifications of the electrical activity of hypothalamic neurones by pineal indoles. Progr. Brain Res., 52, 373-375.

De Mairan, J. (1729). Observation botanique. Histoire de l'Academie Royale des Sciences., pp. 35-36. Paris.

De Prins, J., Cornelissen, G. and Malbecq, W. (1986). Statistical procedures in chronobiology and chronopharmacology. In: Annual Review of Chronopharmacology, Vol. 2. Eds. Reinberg, A., Smolensky, M. and Labrecque, G., pp. 27-141. Oxford: Pergamon.

Derkach, V., Surprenant, A. and North, R.A. (1989). 5-HT$_3$ receptors are membrane ion channels. Nature, 339, 706-709.

De Vivo, M. and Maayani, S. (1985). Inhibition of forskolin-

stimulated adenylate cyclase activity by 5-HT receptor agonists. Eur. J. Pharmacol., 119, 231-234.

De Vivo, M. and Maayani, S. (1986). Characterization of 5-hydroxytryptamine-$_{1A}$-receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea pig and rat hippocampal membranes. J. Pharmacol. Exp. Ther., 238, 248-253.

Dourish, C.T., Hutson, P.H. and Curzon, G. (1985). Low doses of the putative serotonin agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OHDPAT) elicit feeding in the rat. Psychopharmacology, 86, 197-204.

Dourish, C.T., Hutson, P.H. and Curzon, G. (1986a). parachlorophenylalanine prevents feeding induced by the serotonin agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT). Psychopharmacology, 89, 467-471.

Dourish, C.T., Hutson, P.H., Kennett, G.A. and Curzon, G. (1986b). 8-OH-DPAT-induced hyperphagia: Its neural basis and possible therapeutic relevance. Appetite, 7, 127-140.

Dubocovich, M.L. (1983). Melatonin is a potent modulator of dopamine release in the retina. Nature, 306, 782-784.

Dubocovich, M.L. (1985). Characterization of a retinal melatonin receptor. J. Pharmacol. Exp. Ther., 234, 395-401.

Dubocovich, M.L. and Takahashi, J.S. (1987). Use of 2-[^{125}I]-iodomelatonin to characterize melatonin binding sites in chicken retina. Proc. Natl. Acad. Sci. USA., 84, 3916-3920.

Dubocovich, M.L. (1988). Pharmacology and function of melatonin receptors. FASEB J., 2, 2765-2773.

Dugovic, C. and Wauquier, A. (1988). Melatonin modulates the ritanserin-induced sleep changes in the rat. Psychopharmacology, 96 (Suppl), 293.

Dugovic, C., Wauquier, A., Leysen, J.E., Marrannes, R. and Janssen, P.A.J. (1989a). Functional role of 5-HT$_2$ receptors in the regulation of sleep and wakefulness in the rat. Psychopharmacology, 97, 436-442.

Dugovic, C., Leysen, J.E. and Wauquier, A. (1989b). Melatonin modulates the sensitivity of 5-hydroxytryptamine$_2$ receptor-mediated sleep-wakefulness regulation in the rat. Neurosci. Letts., 104, 320-325.

Dumuis, A., Bouhelal, R., Sebben, M., Cory, R. and Bockaert, J. (1988). A nonclassical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system. Mol. Pharmacol., 34, 880-887.

Ebadi, M. (1984). Regulation of the synthesis of melatonin and its significance to neuroendocrinology. In: The Pineal Gland. Ed. Reiter, R.J., pp. 1-38. New York: Raven Press.

Ebadi, M. and Govitrapong, P. (1986). Orphan transmitters and their receptor sites in the pineal gland. Pineal Res. Rev., 4, 1-54.

Eichler, V. and Moore, R.Y. (1971). Pineal hydroxyindole-O-methyl-transferase and gonadal responses to blinding or continuous darkness blocked by pineal denervation in the male hamster. Neuroendocrinology, 8, 81-85.

Elliott, J.A. (1976). Circadian rhythms and photoperiodic time measurement in mammals. Fed. Proc., 35, 2339-2346.

Elliott, J.A., Stetson, M.H. and Menaker M. (1972). Regulation of testis function in golden hamsters: A circadian clock measures photoperiodic time. Science, 171, 1169-1171.

Engel, G., Gothert, M., Muller-Schweinitzer, E., Schlicker, E., Sistonen, L. and Stadler, P.A. (1983). Evidence for common pharmacological properties of [^{3}H]5-hydroxytryptamine binding sites, presynaptic 5-hydroxytryptamine autoreceptors in CNS and inhibitory presynaptic 5-hydroxytryptamine receptors on sympathetic nerves. Naunyn-Schmiedeberg's Arch. Pharmacol., 324, 116-124.

Extance, K. and Goudie, A.J. (1981). Inter-animal olfactory cues in operant drug discrimination procedures in rats. Psychopharmacology, 73, 36-371.

Falcon, J. and Collin, J.P. (1989). Photoreceptors in the pineal of lower vertabrates: Functional aspects. Experientia, 45(10), 909-913.

Fargin, A., Raymond, J.R., Lohse, M.J., Kobilka, B.K., Caron, M.G. and Lefkowitz, R.J. (1988). The genomic clone G-21 which resembles a beta-adrenergic receptor sequence encodes the 5-HT$_{1A}$ receptor. Nature, 335, 358-360.

Farrow, J.T. and Van Vunakis, H. (1973). Characteristics of d-lysergic acid diethylamide binding to subcellular fractions derived from rat brain. Biochem. Pharmacol., 22, 1103-1113.

Fernstrom J.D. (1981). Dietary precursors and brain neurotransmitter formation. Ann. Rev. Med., 32, 413-425.

Fioretti, M.C., Riccardi, C., Menconi, E. and Martini, L. (1974). Control of the circadian rhythm of the body temperature in the rat. Life Sci., 14, 211-219.

Foldes, A., Hoskinson, R.M., Scaramuzzi, R.J., Hinks, N.T. and Maxwell, C.A. (1983). Modification of sheep pineal beta-adrenoceptors by some gonadal steroids but not by melatonin. Neuroendocrinology, 37, 378-385.

Foote, W.E., Taber-Pierce, E. and Edwards, L. (1978). Evidence for a retina projection to the midbrain of the cat. Brain Res., 156, 135-140.

Fowler, C.J. and Tipton, K.F. (1982). Deamination of 5-hydroxytryptamine by both forms of monoamine oxidase in the brain. J. Neurochem., 38, 733-736.

Fozard, J.R. (1987). 5-HT: The Enigma Variations. Trends Pharmacol. Sci., 8, 501-506.

Franey, C., Aldous, A., Burton, S., Checkley, S. and Arendt, J. (1986). Acute treatment with desipramine stimulates melatonin and 6-sulphatoxymelatonin in man. Br. J. Clin. Pharmacol., 22, 73-79.

Friedman, M.K. and Stricker, E.M. (1976). The physiological psychology of hunger: A physiological perspective. Psychol. Rev., 83, 409-431.

Freidman, R., Barrett, R.J. and Sanders-Bush, E. (1983). Additional evidence that L-5-Hydroxytryptophan discrimination models a unique serotonin receptor. Psychopharmacology, 80, 209-213.

Friedman, R.L., Barrett, R.J. and Sanders-Bush, E. (1984). Discriminative stimulus properties of quipazine: Mediation by serotonin2-binding sites. J. Pharmacol. Exp. Ther., 228, 628-635.

Fuxe, K. (1965). Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. Acta. Physiol. Scand., 64 (Suppl. 247), 39-85.

Gaddum, J.H. and Picarelli, Z.P. (1957). Two kinds of tryptamine receptor. Br. J. Pharmacol., 12, 323-328.

Gaffori, O. and Van Ree, J. M. (1985). Serotonin and antidepressant drugs antagonize melatonin-induced behavioural changes after injection into the nucleus accumbens of rats. Neuropharmacology, 24(3), 237-244.

Gal, E.M. and Whitacre, D.H. (1981). Biopterin. VII. Inhibition of synthesis of reduced biopterins and its bearing on the function of cerebral tryptophan-5-hydroxylase *in vivo*. Neurochem. Res., 6, 233-241.

Garrick, N.A., Tamarkin, L., Taylor, P.L. and Markey, S.P., Murphy, D.L. (1983). Light and propranolol suppress the nocturnal elevation of serotonin in the cerebrospinal fluid of rhesus monkeys. Science, 221, 474-476.

Gaston, S. and Menaker, M. (1967). Photoperiodic control of hamster testis. Science, 158, 925-928.

Gerson, S.C. and Baldessarini, R.J. (1980). Motor effects of serotonin in the central nervous system. Life Sci., 27, 1435-1451.

Geyer, M.A. and Light, R.K. (1979). LSD-induced alterations of investigatory responding in rats. Psychopharmacology, 65, 41-47.

Glass, D.J. and Lynch, R.G. (1981). Melatonin: Identification of sites of antigonadal action in mouse brain. Science, 214, 821-823.

Glennon, R.A., Rosecrans, J.A., Young, R. and Gaines, J. (1979). Hallucinogens as discriminative stimuli: generalization of DOM to a 5-methoxy-N,N-dimethyltryptamine stimulus. Life Sci., 24, 93-998.

Glennon, R.A., Young, R. and Rosecrans, J.A. (1982). Discriminative stimulus properties of DOM and several molecular modifications. Pharmacol. Biochem. Behav., 16, 553-556.

Glennon, R.A., Young, R., Jacyno, J.M., Slusher, M. and Rosecrans, J.A. (1983a). DOM-stimulus generalization to LSD and other hallucinogenic indolealkylamines. Eur. J. Pharmacol., 86, 453-459.

Glennon, R.A., Young, R. and Rosecrans, J.A. (1983b). Antagonism of the effects of the hallucinogen DOM and the purported serotonin agonist quipazine by 5-HT_2 antagonists. Eur. J. Pharmacol., 91, 189-196.

Glennon, R.A., McKenney, J.D. and Young, R. (1984a). Discriminative stimulus properties of the serotonin agonist 1-(3-trifluoromethylphenyl) piperazine (TFMPP). Life Sci., 35, 1475-1480.

Glennon, R.A., Titeler, M. and McKenney, J.D. (1984b). Evidence for 5-HT_2 involvement in the mechanism of action of hallucinogenic agents. Life Sci., 35, 2505-2511.

Glennon, R.A., Titeler, M. and Young, R. (1986). Structure-activity relationships and mechanisms of action of hallucinogenic agents based on drug discrimination and ligand-binding studies. Psychopharmacol. Bull., 22, 953-958.

Glennon, R.A., Pierson, M.E. and McKenney, J.D. (1988). Stimulus generalization of TFMPP to propranolol, pindolol and mesulergine. Pharmacol. Biochem. Behav., 29, 197-199.

Goldman, H. and Wurtman, R. (1964). Flow of blood to the pineal body of the rat. Nature, 203, 27-28.

Goldman, B.D., Hall, V., Hollister, C., Roychoudhury, P., Tamarkin, L. and Westrom, W. (1979). Effects of melatonin on the reproductive system in intact and pinealectomized male hamsters maintained under various photoperiods. Endocrinology, 104, 82-88.

Goodwin, G.M. and Green, A.R. (1985). A behavioural and biochemical study in mice and rats of putative agonists and antagonists for 5-HT_1 and 5-HT_2 receptors. Br. J. Pharmacol., 84, 743-753.

Goodwin, G.M., De Souza, R.J. and Green, A.R. (1985a). The pharmacology of the hypothermic response in mice to 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT). Neuropharmacology, 24, 1187-1194.

Goodwin, G.M., De Souza, R.J. and Green, A.R. (1985b). Presynaptic serotonin receptor-mediated response in mice attenuated by antidepressant drugs and electroconvulsive shock. Nature, 317, 531-533.

Goodwin, G.M., De Souza, R.J. and Green, A.R. (1986). The effect of a 5-HT_1 receptor ligand isapirone (TVX Q 7821) on 5-HT synthesis and on the behavioural effects of 5-HT agonists in mice and rats. Psychopharmacology, 89, 382-387.

Goodwin, G.M., De Souza, R.J., Green, A.R. and Heal, D.J. (1987). The pharmacology of the behavioural and hypothermic responses of rats to 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). Psychopharmacology, 91, 506-511.

Goudie, A.J., Thornton, E.W. and Wheeler, T.J. (1976). Effects of Lilly 110140, a specific inhibitor of serotonin uptake, on food uptake and 5-hydroxytryptophan induced anorexia. J. Pharm. Pharmacol., 28, 318-320.

Green, A.R. and Goodwin, G.M., (1987). The pharmacology of the hypothermic response of rodents to 8-OHDPAT administration and the effects of psychotropic drug administration on this response. In: Brain 5-HT$_{1A}$ receptors. Eds. Dourish, C.T., Ahlenius, S. and Hutson, P.H., pp. 161-176. Chichester: Horwood.

Green, A.R., Gillin, J.C. and Wyatt, R.J. (1976). The inhibitory effect of intraventricular administration of serotonin on spontaneous motor activity of rats. Psychopharmacology, 51, 81-84.

Green, A.R., Guy, A.P. and Gardner, C.R. (1984). The behavioural effects of RU 24969, a suggested 5-HT$_1$ agonist in rodents and the effect on the behaviour of treatments with antidepressants. Neuropharmacology, 23, 655-661.

Gudelsky, G.A., Koenig, J.I. and Meltzer, H.Y. (1986). Thermoregulatory responses to serotonin (5-HT) receptor stimulation in the rat. Evidence for opposing roles of 5-HT$_2$ and 5-HT$_{1A}$ receptors. Neuropharmacology, 25(12), 1307-1313.

Gwinner, E. and Benzinger, I. (1978). Synchronization of a circadian rhythm in pinealectomized European starlings by daily injections of melatonin. J. Comp. Physiol., 127, 209-213.

Halberg, F., (1959). Physiologic 24-hour periodicity: General and procedural considerations with reference to the adrenal cycle. Zeitschrift fur Vitamin-, Hormon-, und Fermentforschung, 10, 225-296.

Hamm, H.E. and Menaker, M. (1980). Retinal rhythms in chicks: Circadian variation in melatonin and in serotonin N-acetyltransferase activity. Proc. Natl. Acad. Sci. USA., 77, 4998-5002.

Hamon, M., Cossery, J-M., Spampinato, U. and Gozlan, H. (1986). Are there selective ligands for 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor binding sites in the brain? Trends Pharmacol. Sci., 7, 336-338.

Harrington, M. E. and Rusak, B. (1986). Lesions of the thalamic intergeniculate leaflet alter hamster circadian rhythms. J. Biol. Rhythms, 1, 309-325.

Harrington, M. E., Nance, D.M. and Rusak, B. (1985). Neuropeptide-Y immunoreactivity in the hamster geniculo-suprachiasmatic tract. Brain Res. Bull., 15, 465-472.

Hartig, P.R. (1989). Molecular biology of 5-HT receptors. Trends Pharmacol. Sci., 10, 64-69.

Hastings, M.H., Vance, G. and Maywood, E. (1989). Phylogeny and function of the pineal. Experientia, 45(10), 903-909.

Hedlund, L., Lischko, M.M., Rollag, M.D. and Niswender, G.D. (1977). Melatonin: Daily cycle in plasma and cerbrospinal fluid of calves. Science, 195, 686-687.

Hendel, R.C. and Turek, F.W. (1978). Suppression of locomotor activity in sparrows by treatment with melatonin. Physiol. Behav., 21, 275-278.

Hendrickson, A.E., Wagoner, N. and Cowan, W.M. (1972). An autoradiographic and electron microscopic study of retino-hypothalamic connections. Z. Zellforsch, 135, 1-26.

Herbert, J. (1989). Neural systems underlying photoperiodic time measurement: A blueprint. Experientia, 45(10), 965-972.

Heuring, R.E. and Peroutka, S.J. (1987). Characterization of a novel [^{3}H]-5-hydroxytryptamine binding site subtype in bovine brain membranes. J. Neurosci., 7, 894-903.

Heward, C.G. and Hadley, M.E. (1975). Structure-activity relationships of melatonin and related indoleamines. Life Sci., 17, 1167-1178.

Hewing, M. (1978). A liquor containing area in the pineal recess of the golden hamster (*Mesocricetus auratus*). Anat. Embryol., 153, 295-304.

Hillier, J.G., Davies, J.A. and Redfern, P.H. (1973). An environmental cabinet for 24-hour rhythm research of small animals. J. Interdisciplinary Cycle Res., 4, 79-82.

Hillier, J.G. and Redfern, P.H. (1977). 24-hour rhythm in serum and brain indoleamines: Tryptophan hydroxylase and MAO activity in the rat. Int. J. Chronobiol., 4, 197-210.

Hirata, F., Hayaishi, O., Tokuyama, T. and Seno, S. (1974). *In vitro* and *in vivo* formation of two new metabolites of melatonin. J. Biol. Chem., 249, 1311-1313.

Hirschhorn, I. and Winter, J.C. (1971). Mescaline and lysergic acid diethylamide (LSD) as discriminative stimuli. Psychopharmacologia, 22, 64-71.

Hjorth, S., Carlsson, A., Hindberg, P., Sanchez, D., Wikstrom, H., Arvidsson, L.E., Hacksell, U. and Nilsson, J.L.G. (1982). 8-hydroxy-2-(di-n-propylamino)tetralin, 8-OHDPAT, a potent and selectve simplified ergot congener with central 5-HT receptor stimulating activity. J. Neural Transm., 55, 169-188.

Ho, A.K. and Klein, D.C. (1987). Activation of alpha$_1$-adrenoceptors, protein kinase C or treatment with intracellular free Ca^{2+} elevating agents increases pineal phospholipase A_2 activity. J. Biol. Chem., 262, 11764-11770.

Ho, A.K., Cena, V. and Klein, D.C. (1987a). Cardiac glycosides stimulate phospholipase C activity in rat pinealocytes. Biochem. Biophys. Res. Commun., 142, 819-825.

Ho, A.K., Chik, C.L. and Klein, D.C. (1987b). Transmembrane receptor crosstalk: concurrent VIP and alpha$_1$-adrenergic activation rapidly elevates pinealocyte cGMP >100-fold. Biochem. Biophys. Res. Commun., 146, 1478-1484.

Hoffman, R.A., Johnson, L.B. and Reiter, R.J. (1985). Harderian Glands of golden hamsters: Temporal and sexual differences in immunoreactive melatonin. J. Pin. Res., 2, 161-168.

Holloway, F.A. and Wansley, R.A. (1973a). Multiphasic retention deficits at periodic intervals after passive avoidance learning. Science, 180, 208-210.

Holloway, F.A. and Wansley, R.A. (1973b). Multiple retention deficits at periodic intervals after active and passive avoidance learning. Behav. Biol., 2, 1-14.

Holmes, S.W. and Sugden, D. (1982). Effects of melatonin on sleep and neurochemistry in the rat. Br. J. Pharmacol., 76, 95-101.

Holtzman, S.G. and Locke, K.W. (1988). Neural mechanisms of drug stimuli: experimental approaches. In: Transduction Mechanisms of Drug Stimuli. Eds. Colpaert, F.C. and Balster, R.L., pp. 139-153. Berlin: Springer-Verlag.

Honma, K.I., Katabami, F., Hiroshige, T. (1978). A phase response curve for the locomotor activity rhythm of the rat. Experientia, 34(12), 1602-1603.

Honma, K.I., Watanabe, K. and Hiroshige, T. (1979). Effects of parachorophenylalanine and 5,6-dihydroxytryptamine on the free-running rhythms of locomotor activity and plasma corticosterone in the rat exposed to continuous light. Brain Res., 169, 531-544.

Hoyer, D., Waeber, C., Pazos, A., Probst, A. and Palacios, J.M. (1988). Identification of a 5-HT$_1$ recognition site in human brain membranes different from 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ sites. Neurosci. Letts., 85, 357-362.

Hoyer, D. and Schoeffter, P. (1988). 5-HT$_{1D}$ receptors inhibit forskolin-stimulated adenylate cyclase activity in calf substantia nigra. Eur. J. Pharmacol., 147, 145-147.

Hutson, P.H., Dourish, C.T. and Curzon, G. (1986). Neurochemical and behavioural evidence for mediation of the hyperphagic action of 8-OH-DPAT by 5-HT cell body autoreceptors. Eur. J. Pharmacol., 129, 347-352.

Hutson, P.H., Donohoe, T.P. and Curzon, G. (1988). Infusion of the 5-hydroxytryptamine agonists RU24969 and TFMPP into the paraventricular nucleus of the hypothalamus causes hypophagia. Psychopharmacology, 95, 550-552.

Inouye, S.T. and Kawamura, H. (1979). Persistence of circadian rhythmicity in a mammalian hypothalamic "island" containing the suprachiasmatic nucleus. Proc. Natl. Acad. Sci. USA., 76, 5962-5966.

Iversen, S.D. (1984). 5-HT and Anxiety. Neuropharmacology, 23, 1533-1560.

Jacobs, B.L. (1976). An animal behaviour model for studying central serotonergic synapses. Life Sci., 19, 777-786.

Jarbe, T.U.C. and Swedberg, M.D.B. (1982). A conceptualization of drug discrimination learning. In: Drug Discrimination: Applications in CNS Pharmacology. Eds. Colpaert, F.C. and Slangen, J.L., pp. 327-341. Amsterdam: Elsevier.

Jarbe, T.U.C., Sterner, U. and Hjerpe, C. (1981). Conditioning of an interoceptive drug stimulus to different exteroceptive contexts. Psychopharmacology, 73, 23-26.

Jarbe, T.U.C., Laaksonen. T. and Svensson, R. (1983). Influence of exteroceptive contextual conditions upon internal drug stimulus control. Psychopharmacology, 80, 31-34.

Jarbe, T.U.C. and Johansson, B. (1984). Interaction between drug discriminative stimuli and exteroceptive, sensory signals. Behav. Neurosci., 98(4), 686-694.

John, J.M., Itoh, S. and George, J.C. (1978). On the role of the pineal in thermoregulation in the pigeon. Horm. Res., 9, 41-56.

Johnson, M.S. (1926). Activity and distribution of certain wild mice in relation to biotic communities. J. Mammal 7(4), 245-277.

Johnson, M.S. (1939). Effect of continuous light on periodic spontaneous activity of white-footed mice (*peromyscus*). J. Exp. Zool., 85, 315-328.

Johnston, J.P. (1968). Some observations upon a new inhibitor of monoamine oxidase in brain tissue. Biochem. Pharmacol., 17, 1285-1297.

Julius, D., MacDermott, A.B., Axel, R. and Jessel, T.M. (1988). Molecular characterization of a functional cDNA encoding the serotonin 1C receptor. Science, 241, 558-564.

Kakolewski, J.W., Deaux, E., Christensen, J. and Case, B. (1971). Diurnal patterns in water and food intake and body weight changes in rats with hypothalamic lesions. Am. J. Physiol., 221, 711-718.

Kaku, K., Inoue, Y., Matsutani, A., Okubo, M., Hatao, K., Kaneki, T. and Yanaihara, N. (1983). Receptors for vasoactive intestinal polypeptide on rat dispersed pineal cells. Biomed. Res., 4, 321-328.

Kam, L.M. and Moberg, G.P. (1977). Effect of raphe lesions on the circadian pattern of wheel-running in the rat. Physiol. Behav., 18, 213-217.

Kamberi, I.A. (1974). The role of brain monoamines and pineal indoles in the secretion of gonadotrophins and gonadotrophin-releasing factors. Prog. Brain Res., 41, 261-280.

Kaneko T., Cheng P.Y., Oka H., Oda T., Yanaihara N., Yanaihara C. (1980). Vasoactive intestinal polypeptide stimulate adenylate cyclase and serotonin N-acetyltransferase activities in rat pineal *in vitro*. Biomed. Res., 1, 84-87.

Kappers, J.A. (1983). Innervation of the vertabrate pineal organ. In: The Pineal Gland and its Endocrine Role. Eds. Axelrod, J., Fraschini, F. and Velo, G.P. pp. 87-112. New York: Plenum Press.

Kappers, J.A., Smith, A.R. and De Vries, R.A.C. (1973). The mammalian pineal gland and its control of hypothalamic activity. In: Prog. Brain Res. Vol. 39: Drug Effects on Neuroendocrine Regulation. Eds. Zimmerman, E., Gispen, W.H., Marks, B.H. and De Wied, D., pp. 149-174. Amsterdam: Elsevier.

Kastin, A.J., Miller, J.H., Ferrell, L. and Schally, A.V. (1973). General activity in intact and hypophysectomized rats after administration of melanocyte-stimulating hormone (MSH), melatonin, and Pro-Leu-Gly-NH2. Physiol. Behav., 10, 399-401.

Kennett, G.A. and Curzon, G. (1988a). Evidence that mCPP may have behavioural effects mediated by central 5-HT$_{1C}$ receptors. Br. J. Pharmacol., 94, 137-147.

Kennett, G.A. and Curzon, G. (1988b). Evidence that hypophagia induced by mCPP and TFMPP requires 5-HT$_{1C}$ and 5-HT$_{1B}$ receptors; hypophagia induced by RU24969 only requires 5-HT$_{1B}$ receptors. Psychopharmacology, 96, 93-100.

Kennett, G. A. and Curzon, G. (1988c). The antiemetic drug trimethobenzamide prevents hypophagia due to acetyl salicylate, but not to 5-HT$_{1B}$ or 5-HT$_{1C}$ agonists. Psychopharmacology, 96, 101-103.

Kennett, G.A., Dourish, C.T. and Curzon, G. (1987). 5-HT$_{1B}$ agonists induce anorexia at a postsynaptic site. Eur. J. Pharmacol., 141, 429-435.

Kenny, G.C.T. (1961). The "nervus conarii" of the monkey. (An experimental study). J. Neuropathol. Exp. Neurol., 20, 563-571.

Kilpatrick, G.J., Jones, B.J. and Tyers, M.B. (1987). Identification and distribution of 5-HT$_3$ receptors in rat brain using radioligand binding. Nature, 330, 746-748.

Kincl, F.A., Chang C.C. and Zbuzkova, V. (1970). Observations on the influence of changing photoperiod on spontaneous wheel-running activity of neonatally pinealectomized rats. Endocrinology, 87, 38-42.

King, T.S. and Dougherty, W.J. (1982a). Effect of denervation on "synaptic" ribbon populations in the rat pineal gland. J. neurocytol., 11, 19-28.

King, T.S. and Dougherty, W.J. (1982b). Age related changes in pineal "synaptic" ribbon populations in rats exposed to continuous light or darkness. Am. J. Anat., 163, 169-179.

King, T.S., Steger, R.W., Steinlechner, S. and Reiter, R.J. (1984). Day-night differences in estimated rates of 5-hydroxytryptamine turnover in rat pineal gland. Exp. Brain Res., 54, 432-426.

Kitay, J.I. and Altschule, M.D. (1954). The effects of pineal extract administration on ovary weight in rats. Endocrinology, 55, 782-784.

Klein, D.C. and Berg, G.R. (1970). Pineal gland: Stimulation of melatonin production by norepinephrine involves cyclic AMP mediated stimulation of N-acetyltransferase. Adv. Biochem. Psychopharmacol., 3, 241-263.

Klein, D.C. and Moore, R.Y. (1979). Pineal N-acetyltransferase and hydroxyindole-O-methyltransferase: Control by the retinohypothalamic tract and the suprachiasmatic nucleus. Brain Res., 174, 245-262.

Klein, D.C., Auerbach, D.A. and Weller, J.L. (1981). Seesaw signal processing in pineal cells: homologous sensitization of adrenergic stimulation of cyclic GMP accompanies homologous desensitization of beta-adrenergic stimulation of cyclic AMP. Proc. Natl. Acad. Sci. USA., 78, 4625-4629.

Klein, D.C., Smoot, R. and Weller, J.L. (1983a). Lesions of the paraventricular nucleus of the hypothalamus disrupt the suprachiasmatic to spinal cord circuit in the melatonin rhythm generating system. Brain Res. Bull., 10, 647-652.

Klein, D.C., Sugden, D. and Weller, J.L. (1983b). Postsynaptic alpha-adrenergic receptors potentiate the beta-adrenergic stimulation of pineal serotonin N-acetyltransferase. Proc. Natl. Acad. Sci. USA., 80, 599-603.

Knowles, R.G. and Pogson, C.I. (1984). Tryptophan uptake and hydroxylation in ray forebrain synaptosomes. J. Neurochem., 42, 677-684.

Koek, W. and Slangen, J.L. (1984). External stimulus control in a "drug discrimination" procedure: Drug effects and inter-animal variation. Psychopharmacology, 82, 168-173.

Korf, H. W. and Muller, M. (1984). The innervation of the mammalian pineal gland with special reference to central pinealopetal connections. Pineal Res. Rev., 2, 41-86.

Kovacs, G. L., Gajari, I., Telegdy, G. and Lissak, K. (1974). Effect of melatonin and pinealectomy on avoidance and exploratory activity in the rat. Physiol. Behav., 13, 349-355.

Krimmer, E.C. (1974). Drugs as discriminative stimuli. Dissertation Abstr. Internat., 35, 4572-B.

Kruk, Z.L. and Pycock, C.J. (1983). Neurotransmitters and Drugs. 2nd

Laitinen, J.T., Torda, T. and Saavedra, J.M. (1989). Cholinergic stimulation of phosphoinositide hydrolysis in the rat pineal gland. Eur. J. Pharmacol., 161, 237-240.

Laitinen, J.T., Castre, E., Vakkuri, O. and Saavedra, J.M. (1989). Diurnal rhythm of melatonin binding in the rat suprachiasmatic nucleus. Endocrinology, 124(3), 1585-1587.

Laudon, M., Yaron, Z. and Zisapel, N. (1988). N-(3,5-dinitrophenyl)-5-methoxytryptamine, a novel melatonin antagonist: Effects on sexual maturation of the male and female rat and on the oestrous cycles of the female rat. J. Endocr., 116, 43-53.

Launay, J.M., Lemaitre, B.J., Husson, H.P., Dreux, C., Hartmann, L. and DaPrada, M. (1982). Melatonin synthesis by rabbit platelet. Life Sci., 31, 1487-1494.

Leeb-Lundberg, L.M.F., Cotecchia, S., De Blasi, A., Caron, M.G. and Lefkowitz, R.J. (1986). Regulation of adrenergic receptor function by phosphorylation I. Agonist-promoted desensitization and phosphorylation of alpha$_{1}$-adrenergic receptors coupled to inositol phospholipid metabolism in DDT,MF-2 smooth muscle cells. J. Biol. Chem., 262, 3098-3105.

Lefkowitz, R.J., Benovic, J.L., Kobilka, B. and Caron, M.G. (1986). Beta-adrenergic receptors and rhodopsin: shedding new light on an old subject. Trends Pharmacol. Sci., 7, 444-448.

Leino, M. and Airaksinen, M.M. (1985). Methoxyindoles of the retina. Medical Biology, 63, 160-169.

Le Magnen, J., Devos, M., Gaudilliere, J.P., Louis-Sylvestre, J. and Tallon, S. (1973). Role of a lipostatic mechanism in regulation by feeding of energy balance in rats. J. Comp. Physiol. Psychol., 84, 1-23.

Leonhardt, S. and Titeler, M. (1989). Serotonin 5-HT$_{2}$ receptors: two states versus two subtypes. J. Neurochem., 53, 316-317.

Leonhardt, S., Herrick-Davies, K. and Titeler, M. (1989). Detection of a novel serotonin receptor subtype (5-HT$_{1E}$) in human brain: Interaction with a GTP-binding protein. J. Neurochem., 53, 465-471.

Lerner, A.B., Case, J.D., Takahashi, Y., Lee, T.H. and Mori, W. (1958). Isolation of melatonin, the pineal factor that lightens melanocytes. J. Am. Chem. Soc., 80, 2587.

Lerner, A.B., Case, J.D. and Heinzelman, R.V. (1959). Structure of melatonin. J. Am. Chem. Soc., 81, 6084-5.

Levine, J.D., Rosenwasser, A.M., Yanovski, J.A. and Adler, N.T. (1986). Circadian activity rhythms in rats with midbrain raphe lesions. Brain Res., 384, 240-249.

Lewy, A.J., Wehr, T.A., Goodwin, F.K., Newsome, D.A. and Markey, S.P. (1980). Light suppresses melatonin secretion in humans. Science, 210, 1267-1269.

Lewy, A.J., Kern, H.E., Rosenthal, N.E. and Wehr T.A. (1982). Bright artificial light treatment of a manic-depressive patient with a seasonal mood cycle. Am. J. Psychiatry, 139, 1496-1498.

Leysen, J.E. (1984). Problems in *in vitro* receptor binding studies and identification and role of serotonin receptor sites. Neuropharmacology, 23, 247-254.

Leysen, J.E. (1985). Characterization of serotonin binding sites. In: Neuropharmacology of Serotonin. Ed. Green, A.R., pp. 79-116. Oxford: Oxford University Press.

Leysen, J.E. and Laduron, P.M. (1977). A serotonergic component of neuroleptic receptors. Arch. Int. Pharmacodyn. Ther., 230, 337-339.

Leysen, J.E., Niemegeers, C.J.E., Tollanaere, J.P. and Laduron, P.M. (1978). Serotonergic component of neuroleptic receptors. Nature, 272, 163-166.

Leysen, J.E., Awouters, F., Kennis, L., Laduron, P.M., Vanderberk, J. and Janssen, P.A.J. (1981). Receptor binding profile of R 41468, a novel antagonist at serotonin$_2$ receptors. Life Sci., 28, 1015-1022.

Leysen, J.E., Niemegeers, C.J.E., Van Nueten, J.M. and Laduron, P.M. (1982). ([^{3}H]-ketanserin (R 41468), a selective [^{3}H]-ligand for serotonin$_2$ receptor binding sites. Mol. Pharmacol., 21, 301-314.

Leiberman, H.R. and Lea, A.E. (1988). Melatonin: effects on sleep and behaviour in man. In: Melatonin: Clinical Perspectives. Eds. Miles A., Philbrick D.R.S. and Thompson C., pp. 118-127. Oxford: Oxford University Press.

Lincoln, G. and Kelly, R.W. (1988). Test of a putative melatonin antagonist in the ram. J. Reprod. Fert., 1, abstr. 27.

Lovell, R.A. and Freedman, D.X. (1976). Stereospecific receptor sites for d-lysergic acid diethylamide in rat brain: effects of neurotransmitters, amine antagonists and other psychotropic drugs. Mol. Pharmacol., 12, 620-630.

Lubbert, H., Hoffman, B.J., Snutch, T.P., Van Dyke, T., Levine, A.J., Hartig, P.R., Lester, H.A. and Davidson, N. (1987). cDNA cloning of a serotonin 5HT$_{1c}$ receptor by electrophysiological assays of mRNA-injected Xenopus oocytes. Proc. Natl. Acad. Sci. USA., 84, 4332-4336.

Lucki, I. and Harvey, J.A. (1979). Increased sensitivity to d- and l-amphetamine action after midbrain raphe lesions as measured by locomotor activity. Neuropharmacology, 18, 243-249.

Lucki, I. and Frazer, A. (1982a). Prevention of the serotonin syndrome in rats by repeated administration of monoamine oxidase inhibitors but not tricyclic antidepressants. Psychopharmacology, 77, 205-211.

Lucki, I. and Frazer, A. (1982b). Behavioural effects of indole- and

piperazine-type serotonin receptor agonists. Soc. Neurosci. Abstr., 8, 101.

Lucki, I. and Frazer, A. (1985). Changes in behaviour associated with serotonin receptors following repeated treatment with antidepressant drugs. In: Behavioural Pharmacology: The Current Status. Eds. Seiden, L.S. and Balster, R.L., pp339-351. New York: Alan Liss.

Lucki, I. and Minugh-Purvis, N. (1987). Serotonin-induced headshaking behaviour in rats does not involve receptors located in the frontal cortex. Brain Res., 420, 403-406.

Lucki, I., Nobler, M.S. and Frazer, A. (1984). Differential actions of serotonin antagonists on two behavioural models of serotonin receptor activation in the rat. J. Pharmacol. Exp. Ther., 228, 133-139.

Lynch, G.R., White, E., Grundel, R. and Berger, M.S. (1978). Effects of photoperiod, melatonin administration, and thyroid blocker on spontaneous daily torpor and temperature regulation in the white-footed mouse, *Peromyscus leucopus*. J.Comp. Physol., 125, 157-163.

Lyon, R.A., Davies, K.H. and Titeler, M. (1987). [^{3}H]-DOB labels a guanyl nucleotide sensitive state of cortical 5-HT$_2$ receptors. Mol. Pharmacol., 31, 194-199.

Mansbach, J.S. and Barrett, J.E. (1987). Discriminative stimulus properties of buspirone in the pigeon. J. Pharmacol. Exp. Ther., 240(2), 364-369.

Maeda, K. (1983). Monoaminergic effect on cerebrospinal fluid production. Nihon University Journal of Medicine, 25, 155-174.

Marchbanks, R.M. (1967). Inhibitory effects of lysergic acid diethylamide derivatives and reserpine on 5-HT binding in nerve ending particles. Biochem. Pharmacol., 16, 1971-1979.

Marczynski, T.J., Yamaguchi, N., Ling, G.M. and Grodzinska, L. (1964). Sleep induced by the administration of melatonin (5-methoxy-N-acetyltryptamine) to the hypothalamus in unrestrained cats. Experientia, 20, 435-437.

Markstein, R., Hoyer, D. and Engel, G. (1986). 5-HT$_{1A}$ receptors mediate stimulation of adenylate cyclase in rat hippocampus. Naunyn-Schmiedeberg's Arch. Pharmacol., 333, 335-341.

Marshall, R.W., Sparks, M.G., Davies, J.A. (1985). A microcomputer system for long-term monitoring and analysis of locomotor activity. In: Circadian Rhythms in the Central Nervous System. Eds. Redfern, P.H., Campbell, I.C., Davies, J.A. and Martin, K.F., pp. 263-270. Basingstoke: MacMillan.

Martin, K.F. and Marsden, C.A. (1985). *In vivo* diurnal variations of 5-HT release in hypothalamic nuclei. In: Circadian Rhythms in the Central Nervous System. Eds. Redfern, P.H., Campbell, I.C., Davies J.A. and Martin, K.F., pp. 81-94. Basingstoke: MacMillan.

Maruki, C., Spatz, M., Ueki, Y., Nagatsu, I. and Bembry, J. (1984). Cerebrovascular endothelial cell culture: metabolism and synthesis of 5-hydroxytryptamine. J. Neurochem., 43, 316-319.

Mason, R. and Garrett, J.C. (1990). Are the electrophysiological effects of melatonin in the rat CNS mediated via 5-HT receptors? Br. J. Pharmacol., 99, 917P.

McCord, C.P. and Allen, F.B. (1917). Evidence associating pineal gland function with alterations in pigmentation. J. Exp. Zoology, 23, 207-224.

McKenney, J.D. and Glennon, R.A. (1986). TFMPP may produce its stimulus effects via a 5-HT$_{1B}$ mechanism. Pharmacol. Biochem. Behav., 24, 43-47.

McKernan, R.M., Quirk, K., Jackson, R.G. and Ragan, C. I. (1990). Solubilization of the 5-hydroxytryptamine$_3$ receptor from pooled rat cortical and hippocampal membranes. J. Neurochem., 54, 924-930.

Mefford, I.N. and Barchas, J.D. (1980). Determination of tryptophan and metabolites in rat brain and pineal tissue by reverse-phase high-performance liquid chromatography with electrochemical detection. J Chromat., 181, 187-193.

Mefford, I.N., Chang, P., Klein, D.C., Namboodiri, M.A.A., Sugden, D., and Barchas, J. (1983). Reciprocal day/night relationship between serotonin oxidation and N-acetylation products in the rat pineal gland. Endocrinology, 113, 1582-1586.

Meijer, J.H. and Groos, G.A. (1988). Responsiveness of suprachiasmatic and ventral lateral geniculate neurons to serotonin and imipramine: a micro-iontophoretic study in normal and imipramine-treated rats. Brain Res. Bull., 20, 89-96.

Mendelson, W.B., Gillin, J.C., Pisner, G. and Wyatt, R.J. (1980). Arginine vasotocin and sleep in the rat. Brain Res., 182, 246-249.

Middlemiss, D.N. (1984). 8-Hydroxy-2-(di-n-propylamino) tetralin is devoid of activity at the 5-hydroxytryptamine autoreceptor in the rat brain. Implications for the proposed link between the autoreceptor and and the [^{3}H]-5-HT recognition site. Naunyn-Schmiedeberg's Arch. Pharmacol., 327, 18-22.

Milburn, G.M. and Peroutka, S.J. (1989). Characterization of [^{3}H]-quipazine binding to 5-hydroxytryptamine$_3$ receptors in rat brain membranes. J. Neurochem., 52, 1787-1792.

Milton, A.S. (1978). The hypothalamus and the pharmacology of thermoregulation. In: Pharmacology of the Hypothalamus. Eds. Cox, B., Morris, T.D. and Weston, A.H., pp. 105-136. Basingstoke: MacMillan Press.

Minneman, K.P. and Iversen, L.L. (1976). Diurnal rhythm in rat pineal cyclic nucleotide phosphodiesterase activity. Nature, 260, 59-61.

Mitchell, P.J. (1989). PhD Thesis: Effect of antidepressant treatment on social behaviour and circadian rhythms of locomotor activity in the rat. University of Bath.

Mittman, S.M. and Geyer, M.A. (1989). Effects of 5-HT$_{1A}$ agonists on locomotor and investigatory behaviours in rats differ from those of hallucinogens. Psychopharmcology, 98, 321-329.

Moller, M., Van Deurs, B. and Westergaard, E. (1978). Vascular permeability to proteins and peptides in the mouse pineal gland. Cell Tissue Res., 195, 1-15.

Moore, R.Y. (1973). Retinohypothalamic projection in mammals. A comparative study. Brain Res., 49, 403-409.

Moore, R.Y. (1978). The innervation of the mammalian pineal gland. In: The Pineal Gland and Reproduction. Ed. Reiter, R.J., pp. 1-29. Basel: Karger.

Moore, R.Y. (1982). The suprachiasmatic nucleus and the organization of a circadian system. Trends Neurosci. 5, 404-407.

Moore, R.Y. (1983). Organization and function of a central nervous system circadian oscillator: The suprachiasmatic hypothalamic nucleus. Fed. Proc., 42, 2783-2789.

Moore, R.Y. and Rapport, R.L. (1971). Pineal and gonadal function in the rat following cervical sympathectomy. Neuroendocrinology, 7, 361-374.

Moore, R.Y. and Eichler, V.B. (1972). Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res., 42, 201-206.

Moore, R.Y. and Lenn, N. J. (1972). A Retinohypothalamic projection in the rat. J. Comp. Neurol., 146, 1-14.

Moore, R.Y., Halaris, A.E. and Jones, B.A. (1978). Serotonin neurons of the midbrain raphe: Ascending projections. J. Comp. Neurol., 180, 417-438.

Morgan, P.J. and Williams, L.M. (1989). Central melatonin receptors: Implications for a mode of action. Experientia, 45(10), 955-965.

Morgan, P.J., Lawson, W., Davidson, G. and Howell, H.E. (1989). Guanine nucleotides regulate the affinity of melatonin receptors on the ovine pars tuberalis. Neuroendocrinology, 50, 359-362.

Morrissey, J.J. and Lovenberg, W. (1978a). Synthesis of RNA in the pineal gland during N-acetyltransferase induction. Biochem. Pharmacol., 27, 551-555.

Morrissey, J.J. and Lovenberg, W. (1978b). Synthesis of RNA in the pineal gland during N-acetyltransferase induction: the effects of actinomycin D, alpha-amanitin, and cordycepin. Biochem. Pharmacol., 27, 557-562.

Moser, P. and Redfern, P.H. (1985a). Circadian variation in behavioural responses to central 5-HT receptor stimulation in the mouse. Psychopharmacology, 86, 223-227.

Moser, P. and Redfern, P.H. (1985b). The discriminative stimulus properties of 5-HTP are mediated by the 5-HT1 receptor. Br. J. Pharmacol., 84, 70P.

Moser, P. (1986). PhD Thesis: The 24-hour variation in behavioural responses to 5-HT receptor stimulation. University of Bath.

Mosko, S.S. and Moore, R.Y. (1977). Neonatal suprachiasmatic nucleus lesions in the rat: Effects on the development of circadian rhythmicity and the retinohypothalamic projection. Soc. Neurosci. Abstr., 3, 352.

Mrosovsky, N. (1988). Phase response curves for social entrainment. J. Comp. Physiol., 162, 35-46.

Mrosovsky, N. and Salmon P.A. (1987). A behavioural method for accelerating re-entrainment of rhythms to new light-dark cycles. Nature, 330, 372-373.

Murakami, N., Hayafuji, C., Sasak, Y., Yamazaki, J. and Takahashi, K. (1983). Melatonin accelerates the re-entrainment of the circadian adrenocortical rhythm in inverted illumination cycle. Neuroendocrinology, 36, 385-391.

Namboodiri, M.A.A., Sugden, D., Klein, D.C. and Mefford, I.N. (1983). 5-hydroxytryptophan elevates serum melatonin. Science, 221, 659-661.

Neff, N.H., Yang, H.T., Goridis, C. and Bialek, D. (1974). The metabolism of indolealkylamines by type A and type B monoamine oxidase of brain. In: Adv. Biochem. Psychopharmacol. Vol. 11: Serotonin - New Vistas. Eds. Costa, E., Gessa, G.L. and Sandler, M., pp. 51-58. New York: Raven Press.

Nelson, D.L., Herbet, A., Enjalbert, A., Bockaert, J. and Hamon, M. (1980). Serotonin-sensitive adenylate cyclase and [^{3}H]-serotonin binding sites in the CNS of the rat. I. Kinetic parameters and pharmacological properties. Biochem. Pharmacol., 29, 2445-2453.

Niemegeers, C.J.E., Colpaert, F.C., Leysen, J.E., Awouters, F. and Janssen, P.A.J. (1983). Mescaline-induced heatwitches in the rat: an *in vivo* method to evaluate serotonin S2 antagonists. Drug Develop Res., 3, 123-125.

Niles, L.P., Wong, Y., Mishra, R.K. and Brown G. (1979). Melatonin receptors in brain. Eur. J. Pharmacol. 55, 219-220.

Oberlander, C., (1982). Effects of a potent 5-HT agonist, RU 24969, on the mesocorticolimbic and nigrostriatal systems. Br. J. Pharmacol., 80, 675P.

O'Dea, R.F. and Zatz, M. (1976). Catecholamine-stimulated cyclic GMP

accumulation in the rat pineal: Apparent presynaptic site of action. Proc. Natl. Acad. Sci. USA., 73, 3398-3402.

Ogle, T.F. and Kitay, J.I. (1977). Effects of melatonin and an aqueous pineal extract on adrenal secretion of reduced steroid metabolites in female rats. Neuroendocrinology, 23, 113-120.

Ogren, S.O., Fuxe, K., Agnati, L.F., Gustafsson, J.A., Jonsson, G. and Holm, A.C. (1979). Reevaluation of the indoleamine hypothesis of depression. Evidence for a reduction of functional activity of central 5-HT systems by antidepressant drugs. J. Neural Transm., 46, 85-103.

Overton, D.A. (1961). Discriminative behaviour based on the presence or absence of drug effects. Am. Psychologist, 16, 453.

Overton, D.A. (1966). State-dependent learning produced by depressant and atropine-like drugs. Psychopharmacologia, 10, 6-31.

Overton, D.A., (1982). Comparison of the degree of discriminability of various drugs using the T-maze drug discrimination paradigm. Psychopharmacology, 76, 385-395.

Palacios, J.M., Pazos, A. and Hoyer, D. (1987). Characterization and mapping of 5-HT_{1A} sites in the brain of animals and man. In: Brain 5-HT_{1A} Receptors. Eds. Dourish, C.T., Ahlenius, S. and Hutson, P.H., pp. 67-81. Chichester: Horwood.

Pang, S.F., Yip, M.K., Lui, H.W., Brown, G.M. and Tsiu, H.W. (1980). Diurnal rhythms of immunoreative N-acetylserotonin and melatonin in the serum of male rats. Acta Endocrinologia, 95, 571-576.

Parfitt, A., Weller, J.L. and Klein, D.C. (1976). Beta adrenergic blockers decrease adrenergically stimulated N-acetyltransferase activity in pineal glands in organ culture. Neuropharmacology, 15, 353-358.

Pazos, A. and Palacios, J.M. (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. Brain Res., 346, 205-230.

Pazos, A., Hoyer, D. and Palacios, J.M. (1984). The binding of serotonergic ligands to the porcine choroid plexus: Characterization of a new type of serotonin recognition site. Eur. J. Pharmacol., 106, 539-546.

Pedigo, N.W., Yamamura, H.I. and Nelson, D.L. (1981). Discrimination of multiple [^{3}H]-5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. J. Neurochem., 36, 220-226.

Peroutka, S.J. (1990). States and subtypes of the 5-HT_2 serotonin receptor: Interpretation of the data. J. Neurochem., 54, 1085-1086.

Peroutka, S.J. and Hamik, A. (1988). [^{3}H]-Quipazine labels 5-HT_3 recognition sites in rat cortical membranes. Eur. J. Pharmacol., 148, 297-299.

Peroutka, S.J. and Snyder, S.H. (1979). Multiple serotonin receptors: Differential binding of ^{3}H-5-hydroxytryptamine, ^{3}H-lysergic acid diethylamide and ^{3}H-spiroperidol. Mol. Pharmacol., 16, 687-699.

Peroutka, S.J. and Snyder, S.H. (1980). Long-term antidepressant treatment decreases spiroperidol-labeled serotonin receptor binding. Science, 210, 88-90.

Peroutka, S.J., Lebovitz, R.M. and Snyder, S.H. (1979). Serotonin receptor binding sites affected differentially by guanine nucleotides. Mol. Pharmacol., 16, 700-708.

Peroutka, S.J., Lebovitz, R.M. and Snyder, S.H. (1981). Two distinct central serotonin receptors with different physiological functions. Science, 212, 827-829.

Pickard, G.E. (1982). The afferent connections of the suprachiasmatic nucleus of the golden hamster with emphasis on the retinohypothalamic projection. J. Comp. Neurol., 211, 65-83.

Pickard, G.E. (1985). Bifurcating axons of retinal ganglion cells terminate in the hypothalamic suprachiasmatic nucleus and the intergeniculate leaflet of the thalamus. Neurosci. Lett., 55, 211-217.

Pickering, D.S. and Niles, L. (1990). Pharmacological characterization of melatonin binding sites in Syrian hamster hypothalamus. Eur. J. Pharmacol., 175, 71-77.

Pierce, P.A. and Peroutka, S.J. (1989). Evidence for distinct 5-hydroxytryptaminez binding site subtypes in cortical membrane preparations. J. Neurochem., 52, 656-658.

Pittendrigh, C.S. (1954). On temperature independence in the clock system controlling emergence time in Drosophila. Proc. Natl. Acad. Sci. USA., 40, 1018-1029.

Pittendrigh, C.S. and Daan, S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents. V. Pacemaker structure: A clock for all seasons. J. Comp. Physiol., 106, 333-335.

Pritchett, D.R., Bach, A.W.J., Wozny, M., Taleb, O., Dal Toso, R., Shih, J.C. and Seeburg, P. (1988). Structure and functional expression of cloned rat serotonin 5-HT$_2$ receptor. EMBO J., 7, 4135-4140.

Puchalski, W. and Lynch, G.R. (1988). Daily melatonin injections affect the expression of circadian rhythmicity in Djungarian hamsters kept under a long-day photoperiod. Neuroendocrinology, 48, 280-286.

Quay, W.B. (1965). Retinal and pineal hydroxyindole-O-methyl-transferase activity in vertabrates. Life Sci., 4, 983-991.

Quay, W.B. (1970a). Physiological significance of the pineal during adaption to shifts in photoperiod. Physiol. Behav., 5, 353-360.

Quay, W.B. (1970b). Precocious entrainment and associated characteristics of activity patterns following pinealectomy and reversal of photoperiod. Physiol. Behav., 5, 1281-1290.

Quay, W.B. (1972). Pineal homeostatic regulation of shifts in the circadian activity rhythm during maturation and aging. N.Y. Acad. Sci., 34, 239-254.

Quay, W.B. and Ma, Y.H. (1976). Demonstration of gastrointestinal-O-methyltransferase. International Reports on Clinical Sciences, Medical Science, 4, 563.

Raikhlin, N.T., Kvetnoy, I.M. and Tolkachev, V.N. (1975). Melatonin may be synthesized in enterochromaffin cells. Nature, 219, 344.

Ralph, C.L., Mull, D., Lynch, H.J. and Hedlund, L. (1971). A melatonin rhythm persists in rat pineals in darkness. Endocrinology, 89, 1361-1366.

Rapport, M.M. (1949). Serum vasoconstrictor (serotonin) v. the presence of creatinine in the complex: A proposed study of the vasoconstrictor principle. J. Biol. Chem., 180, 961-969.

Rapport, M.M., Green, A.A. and Page, I.H. (1948). Crystalline serotonin. Science, 108, 329-330.

Redman, J.R., Armstrong, S.M. and Ng, K.T. (1983). Free-running activity rhythms in the rat: Entrainment by melatonin. Science, 219, 1089-1091.

Redman, J.R. and Armstrong, S.M. (1988). Reentrainment of rat circadian activity rhythms: Effects of melatonin. J. Pin. Res., 5, 203-215.

Reiter, R.J. (1980). The pineal and its hormones in the control of reproduction in mammals. Endocr. Rev., 1, 109-131.

Reiter, R.J. (1981). The mammalian pineal gland: Structure and function. Am. J. Anat., 162, 287-313.

Reiter, R.J. (1983a). The role of light and age in determining melatonin production in the pineal gland. In: The Pineal Gland and its Endocrine Role. Eds. Axelrod J., Fraschini F. and Velo G.P., pp. 227-241. New York: Plenum Press.

Reiter, R.J. (1983b). Melatonin as the hormone which mediates the effects of the pineal gland on neuroendocrine-reproductive axis of the Syrian hamster. In: The Pineal Gland and its Endocrine Role. Eds. Axelrod J., Fraschini F. and Velo G.P., pp. 317-330. New York: Plenum Press.

Reiter, R.J. (1985). Action spectra, dose-response relationship, and temporal aspects of light's effects on the pineal gland. Ann. N.Y. Acad. Sci., 453, 215-230.

Reiter, R.J. (1986). Normal patterns of melatonin levels in the

pineal gland and body fluids of humans and experimental animals. J. Neural Transm, (Suppl.), 21, 35-54.

Reiter, R.J. (1987). The melatonin message: Duration versus coincidence hypothesis. Life Sci., 40, 2119-2131.

Reiter, R.J. (1988). Neuroendocrinology of melatonin. In: Melatonin - Clinical Perspectives. Eds. Miles A., Philbrick D.R.S. and Thompson, C., pp. 1-42. New York: Oxford University Press.

Reiter, R.J., Vaughan, M.K., Blask, D.E. and Johnson, L.Y. (1974). Melatonin: Its inhibition of pineal antigonadotrophic activity in male hamsters. Science, 185, 1167-1169.

Reiter, R.J., Welsh, M.G. and Vaughan, M.K. (1976). Age-related changes in the intact and sympathetically denervated gerbil pineal gland. Am. J. Anat., 146, 427-432.

Reiter, R.J., Rudeen, P.K., Sackman, J.W., Vaughan, M.K., Johnson, L.Y. and Little, J.C. (1977). Subcutaneous melatonin implants inhibit reproductive atrophy in male hamsters induced by daily melatonin injections. Endocr. Res. Commun., 4, 35-44.

Reiter, R.J., Richardson, B.A. and Hurlbut, E.C. (1981). Pineal, retinal and Harderian gland melatonin in a diurnal species, the Richardson's ground squirrel (*Spermophilus richardsonii*). Neurosci. Lett., 22, 285-288.

Reiter, R.J., Richardson, B.A., Matthews, S.A., Lane, S.J. and Ferguson, B.N. (1983). Rhythms in immunoreactive melatonin in the retina and Harderian glands of rats: Persistence after pinealectomy. Life Sci., 32, 1229-1236.

Relkin, R. (1983). Pineal-hormone interactions. In: The Pineal Gland. Ed. Reiter, R.J., pp. 225-246. New York: Elsevier.

Richter, C.P. (1922). A behaviouristic study of the activity of the rat. In: Comparative Psychology Monographs Vol. 1 (serial no. 2). Ed. Hunter W.S., pp. 1-55. Baltimore: Williams and Wilkins Co.

Richter, C.P. (1965). Biological Clocks in Medicine and Psychiatry. Ed. Thomas C.C., Illinois: Springfield.

Richter, C.P. (1967). Sleep and activity: Their relation to the 24-hour clock. Proc. Assoc. Res. Nerv. Ment. Dis., 45, 8-27.

Riley, J.N., Card, J.P. and Moore, R.Y. (1981). A retinal projection to the lateral hypothalamus in the rat. Cell Tissue Res., 214 257-269

Roberts, M.H.T. (1984). 5-Hydroxytryptamine and antinociception. Neuropharmacology, 23, 1529-1536.

Roberts, M.H.T. and Straughan, D.W. (1967). Excitation and depression of cortical neurons by 5-hydroxytryptamine. J. Physiol., 193, 269-294.

Rollag, M.D. and Niswender, G.D. (1976). Radioimmunoassay of serum concentrations in sheep exposed to different lighting regimes. Endocrinology, 98, 482-489.

Rollag, M.D., Morgan, R.J. and Niswender, G.D. (1978). Route of melatonin secretion in sheep. Endocrinology, 102, 1-8.

Romero, J.A., Zatz, M. and Axelrod, J.A. (1975). Beta-aderenergic stimulation of pineal N-acetyltransferase: Adenosine 3',5'-monophosphate stimulates both RNA and protein synthesis. Proc. Natl. Acad. Sci. USA., 72, 2107-2111.

Romijn, H.J. (1973). Parasympathetic innervation of the rabbit pineal gland. Brain Res., 55, 431-436.

Romijn, H.J. (1975). The ultrastructure of the rabbit pineal gland after sympathectomy, parasympathectomy, continuous illumination, and continuous darkness. J. Neural Transm., 36, 183-194.

Romijn, H.J. (1976). The influence of some sympatholytic, parasympatholytic and serotonin-synthesizing-inhibiting agents on the ultra-structure of the rabbit pineal organ. Cell Tissue Res., 167, 167-177

Rosecrans, J.A. and Glennon, R.A. (1979). Drug-induced cues in studying mechanisms of drug action. Neuropharmacology, 18, 981-989.

Rosecrans, R.A., Krynock, G.M., Newlon, P.G., Chance, W.T., and Kallman M.J. (1978). Central mechanisms of drugs as discriminative stimuli involvement of serotonin pathways. In: Stimulus properties of drugs: ten years of progress. Eds. Colpaert, F.C. and Rosecrans, J.A., pp. 83-98. Janssen Research Foundation.

Rosengarten, H., Meller, E. and Friedhoff, A.J. (1972). *In vitro* enzymatic formation of melatonin by human erythrocytes. Res. Comm. Chem. Path. Pharmacol., 4, 457-465.

Rowland, N. (1976). Circadian rhythms and partial recovery of regulatory drinking in rats after lateral hypothalamic lesions. J. Comp. Physiol. Psychol., 90, 382-393.

Rusak, B. (1977). Involvement of the primary optic tracts in mediation of light effects on hamster circadian rhythms. J. Comp. Physiol., 118, 165-172.

Rusak, B. and Zucker, I. (1979). Neural regulation of circadian rhythms. Physiol. Rev., 59(1), 449-526.

Rusak, B. and Groos, G. (1982). Suprachiasmatic stimulation phase shifts rodent circadian rhythms. Science, 215, 1407-1409.

Saavedra, J.M., Brownstein, M. and Axelrod, J. (1973). Specific and sensitive enzymatic isotopic microassay for serotonin in tissues. J. Pharmacol. Exp. Ther., 186, 508-515.

Samanin, R., Bendotti, C., Miranda, F. and Garattini, S. (1977). Decrease of food intake by quipazine in the rat: Relation to

serotonergic receptor stimulation. J. Pharm. Pharmacol., 29, 53-54.

Sanders-Bush, E. and Breeding, M. (1988). Putative selective 5-HT2 antagonists block serotonin 5-HT1c receptors in the choroid plexus. J. Pharmacol. Exp. Ther., 247, 169-173.

Satake, N., Shibata, S. and Takagi, T. (1986). The inhibitory action of melatonin on the contractile response to 5-hydroxytryptamine in various isolated vascular smooth muscles. Gen. Pharmacol., 17(5), 553-558.

Schnellmann, R.G., Waters, S.J. and Nelson, D.L. (1984). [^{3}H]-5-hydroxytrytamine binding sites: Species and tissue variation. J. Neurochem., 42, 65-70.

Schoeffter, P. and Hoyer, D. (1989). 5-Hydroxytryptamine 5-HT1B and 5-HT1D receptors mediating inhibition of adenylate cyclase activity. Pharmacological comparison with special reference to the effects of yohimbine, rauwolscine and some beta-adrenoceptor antagonists. Naunyn-schmiedeberg's Arch Pharmacol., 340, 285-292.

Schrier, B.K. and Klein, D.C. (1974). Absence of choline acetyltransferase in rat and rabbit pineal gland. Brain Res. 79, 347-351.

Schwartz W., Gainer H. (1977). Suprachiasmatic nucleus: Use of [^{14}C]-labeled deoxyglucose uptake as a functional marker. Science, 197, 1089-1091.

Schwartz, W., Davidsen, L. and Smith, C. (1980). *In vivo* metabolic activity of a putative circadian oscillator, the rat suprachiasmatic nucleus. J. Comp. Neurol., 189, 157-167.

Shannon, H.E. and Holtzman, S.G. (1977). Discriminative effects of morphine administered intracerebrally in the rat. Life Sci., 21, 585-594.

Shein, H.M. and Wurtman, R.J. (1971). Stimulation of [^{14}C]-tryptophan 5-hydroxylation by norepinephrine and dibutyryl adenosine 3',5'-mono-phosphate in rat pineal organ cultures. Life Sci. 10, 935-940.

Shenker, A., Maayani, S., Weinstein, H., Green, J.P. (1985). Two 5-HT recptors linked to adenylate cyclase in guina pig hippocampus are discriminated by 5-carboxamidotryptmine and spiperone. Eur. J. Pharmacol., 109, 427-429.

Shenker, A., Maayani, S., Weinstein, H. and Green J.P. (1987). Pharmacological characterization of two 5-hydroxytryptamine receptors coupled to adenylate cyclase in guinea pig hippocampal membranes. Mol. Pharmacol., 31, 357-367.

Shibuya, H., Toru, M., Watanabe, S. (1978). A circadian rhythm of tryptophan hydroxylase in rat pineals. Brain Res., 138, 364-368.

Silverman, A.P. (1978). Animal behaviour in the laboratory. Chapman and Hall, London.

Simonnet, H., Thieblot, L. and Melik, T. (1951). Influence de l'epiphyse sur l'ovaire de la jeunne ratte. Ann. Endocrinol. (Paris), 12, 202-205.

Sitaram B.R., Lees G.J. (1978). Diurnal rhythm and turnover of tryptophan hydroxylase in the pineal gland of the rat. J. Neurochem., 31, 1021-1026.

Sleight, A.J., Smith, R.J., Marsden, C.A. and Palfreyman, M.G. (1988). Are the hyper- and hypophagic responses to 8-OH-DPAT mediated by the same neuronal mechanism? Br. J. Pharmacol., 95, 783P.

Steinlechner, S., Steger, R.W., King, T.S. and Reiter R.J. (1983). Diurnal variation in the serotonin content and turnover in the pineal gland of the Syrian hamster. Neurosci. Letts., 35, 167-172.

Stephan, F.K. (1984). Phase shifts in circadian rhythms of activity entrained to food access. Physiol. Behav., 32(4), 663-671.

Stephan, F.K. (1989). Entrainment of activity to multiple feeding times in rats with suprachiasmatic lesions. Physiol. Behav., 46, 489-497.

Stephan, F.K. and Zucker, I. (1972). Circadian rhythms in drinking behaviour and locomotor activity of rats are eliminated by hypothalamic lesions. Proc. Natl. Acad. Sci. USA., 69, 1583-1586.

Stephan, F.K. and Becker, G. (1989). Entrainment of anticipatory activity to various durations of food access. Physiol. Behav., 46, 731-741.

Stetson, M.H. and Watson-Whitmyre M. (1984). Physiology of the pineal and its hormone melatonin in annual reproduction in rodents. In: The Pineal Gland. Ed. Reiter R., pp. 109-153. New York: Raven Press.

Stolerman, I.P., Garcha, H.S., Pratt, J.A. and Kumar, R. (1984). Role of training dose in discrimination of nicotine and related compounds by rats. Psychopharmacology, 84, 413-419.

Stolerman, I.P., Rauch, R.J. and Norris, E.A. (1987). Discriminative stimulus effects of a nicotine-midazolam mixture in rats. Psychopharmacology, 93, 250-256.

Stolerman, I.P., Stephenson J.D. and Rauch, R.J. (1987). Classification of opioid and 5-hydroxytryptamine receptors by means of discriminative drug effects. Neuropharmacology, 26(7B), 867-875.

Strange, P.G. (1990). States and subtypes of the 5-HT_2 serotonin receptor: Interpretation of the data. J. Neurochem., 54, 1085.

Sugden, D. (1979). Circadian change in rat pineal tryptophan content: Lack of correlation with serum tryptophan. J. Neurochem., 33, 811-813.

Sugden, D. (1980). PhD Thesis: Actions of melatonin on the central nervous system. CNNA: London.

Sugden, D. (1989). Melatonin biosynthesis in the mammalian pineal gland. Experientia, 45(10), 922-932.

Sugden, D. and Klein, D.C. (1983a). Regulation of rat pineal hydroxyindole-O-methyltransferase in neonatal and adult rats. J. Neurochem., 40, 1647-1653.

Sugden, D. and Klein, D.C. (1983b). Adrenergic stimulation of rat pineal hydroxyindole-O-methyltransferase. Brain Res., 265, 348-351.

Sugden, D. and Klein, D.C. (1983c). beta-adrenergic receptor control of rat pineal hydroxyindole-O-methyltransferase. Endocrinology, 113, 348-353.

Sugden, D. and Klein, D.C. (1984). Rat pineal alpha$_1$-adrenoceptors: Identification and characterization using (^{125}I)-iodo-2(b-(4-hydroxyphenyl)ethylaminomethyl)tetralone (^{125}I-HEAT). Endocrinology, 114, 435-440.

Sugden, D., Namboodiri, M.A.A., Klein, D.C., Grady, R. Jr. and Mefford, I.N. (1985a). Ovine pineal indoles: Effects of L-tryptophan or L-5-hydroxytryptohan administration. J. Neurochem., 44, 769-772.

Sugden, D., Namboodiri, M.A.A., Klein, D.C., Pierce, J.E., Grady, R. Jr. and Mefford, I.N. (1985b). Ovine pineal alpha$_1$-adrenceptors: Characterization and evidence for a functional role in the regulation of serum melatonin. Endocrinology, 116, 960-967.

Sugden, D., Vanecek, J., Klein, D.C., Thomas, T.P. and Anderson, W.B. (1985c). Activation of protein kinase C potentiates isoprenaline-induced cyclic AMP accumulation in rat pinealocytes. Nature, 314, 359-361.

Sugden, A.L., Sugden, D. and Klein, D.C. (1987). alpha$_1$-Adrenoceptor activation elevates cytosolic calcium in rat pinealocytes by increasing net influx. J. Biol. Chem., 262, 960-967.

Sugden, D. and Klein D.C. (1988). Activators of protein kinase C act at a post-receptor site to amlify cyclic AMP production in rat pinealocytes. J. Neurochem., 50, 149-155.

Sugden, D., Ho, A.K., Sugden, A.L. and Klein, D.C. (1988). Negative feedback mechanisms: Evidence that desensitzation of pineal alpha$_1$-adrenergic responses involves protein kinase C. Endocrinology, 123, 1423-1432.

Sulser, F. (1983). Mode of action of antidepressant drugs. J. Clin. Psychiatry, 44, 14-20.

Sulser, F., Vetulani, J. and Mobley, P.J. (1978). Mode of action of antidepressant drugs. Biochem. Pharmacol., 27, 257-261.

Sumner, M.J. and Humphrey, P.P.A. (1989). 5-HT$_{1D}$ binding sites in porcine brain can be sub-divided by GR43175. Br. J. Pharmacol., 98,

29-31.

Swanson, L.W., Cowan, W.M., Jones, E.G. (974). An autoradiographic study of the efferent connections of the ventral geniculate nucleus in the albino rat and the cat. J. Comp. Neurol., 56, 143-164.

Tamarkin, L., Westrom, W.K., Hamill, A.I. and Goldman, B.D. (1976). Effect of melatonin on the reproductive systems of male and female hamsters: A diurnal rhythm in sensitivity to melatonin. Endocrinology, 99, 1534-1541.

Taylor, R.L., Alberquerque, M.L.C. and Burt, D.R. (1980). Muscarinic receptors in pineal. Life Sci., 26, 2195-2200.

Taylor, P.A., Garrick, N.A., Tamarkin, L., Murphy, D.L. and Markey, S.P. (1985). Diurnal rhythms of N-acetylserotonin and serotonin in cerebrospinal fluid of monkeys. Science, 228, 900.

Tomie, A., Peoples, L. and Wagner, G.C. (1987). Effects of single or multiple choice trials per session on drug discrimination performance. Psychopharmacology, 92, 529-535.

Trentini, G.P., Mess, B., de Gaetani, C.F. and Ruzsas, C. (1979). Pineal-brain relationships. Prog. Brain Res., 52, 341-366.

Tricklebank, M.D., Forler, C. and Fozard, J.R. (1984). The involvement of subtypes of the 5-HT$_1$ receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-n-propylamino)tetralin in the rat. Eur. J. Pharmacol., 106, 271-282.

Tricklebank, M.D., Middlemiss, D.N. and Neill, J. (1986). Pharmacological analysis of the behavioural and thermoregulatory effects of the putative 5-HT$_1$ receptor agonist, RU-24969, in the rat. Neuropharmacology, 25, 877-886.

Tricklebank, M.D., Neill, J., Kidd, E.J. and Fozard, J.R. (1987). Mediation of the discriminative stimulus properties of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OHDPAT) by the putative 5-HT$_{1A}$ receptor. Eur. J. Pharmacol., 133, 47-56.

Turek, F.W. (1987). Pharmacological probes of the mammalian circadian clock: use of the PRC approach. Trends Pharmacol. Sci., 8, 212-217.

Turek, F.W. and Losee-Olson, S. (1986). A benzodiazepine used in the treatment of insomnia phase-shifts the mammalian circadian clock. Nature, 321, 167-168.

Turek, F.W. and Van Reeth, O. (1988). Altering the mammalian circadian clock with the short-acting benzodiazepine, triazolam. Trends Neurosci., 11(12), 535-541.

Turek, F.W., Desjardins, C. and Menaker, M. (1975). Melatonin antigonadal and progonadal effects in male golden hamsters. Science, 190, 280-282.

Turek, F.W., Desjardins, C. and Menaker M. (1976a). Melatonin-induced inhibition of testicular function in adult golden hamsters. Proc. Soc. Exp. Biol. Med., 151, 502-506.

Turek, F.W., McMillan, J.P. and Menaker, M. (1976b) Melatonin: Effects on the circadian locomotor rhythm of sparrows. Science, 194, 1441-1443.

Twarog, B.M. and Page, I.H. (1953). Serotonin content of some mammalian tissues and urine and a method for its determination. Am. J. Physiol., 175, 157-161.

Uddman, R., Alumets, J., Hakanson, R., Loren, I. and Sundler, F. (1980). Vasoactive intestinal peptide (VIP) occurs in nerves of the pineal gland. Experientia, 36, 1119-1120.

Underwood, H. (1979). Melatonin affects circadian rhythmicity in lizards. J. Comp. Physiol., 130, 317-323.

Underwood, H. (1981). Circadian organization in the lizard *Sceloporus occidentalis*: The effect of pinealectomy, binding and melatonin. J. Comp. Physiol., 141, 537-547.

Underwood, H. (1986). Circadian rhythms in lizards: Phase response curve for melatonin. J. Pin. Res., 3, 187-196.

Underwood, H. and Harless, M. (1985). Entrainment of the circadian activity rhythm of a lizard to melatonin injections. Physiol. Behav., 35, 267-270.

Vacas, M.I., Sarmiento, I.K. and Cardinali, D.P. (1985). Interaction between beta- and alpha-adrenoceptors in rat pineal adenosine cyclic 3',5'-monophosphate and phosphodiesterase activation. J. Neural Transm., 26, 295-304.

Van Reeth, O. and Turek, F.W. (1989a). Stimulated activity mediates phase-shifts in the hamster circadian clock induced by dark pulses or benzodiazepines. Nature, 339, 49-51.

Van Reeth, O. and Turek, F.W. (1989b). Administering triazolam on a circadian basis entrains the activity rhythm of hamsters. Am. J. Physiol., 256, R639-R645.

Vanecek, J. (1988). Melatonin binding sites. J. Neurochem., 51, 1436-1440.

Vanecek, J. and Jansky, L. (1989). Short days induce changes in specific melatonin binding in hamster median eminence and anterior pituitary. Brain Res., 477, 387-390.

Vanecek, J., Sugden, D., Weller, J.L. and Klein, D.C. (1985). Atypical synergistic alpha$_{1}$- and beta-adrenergic regulation of adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate in rat pinealocytes. Endocrinology, 116, 2167-2173.

Vanecek, J., Sugden, D., Weller, J.L. and Klein, D.C. (1986). Seesaw signal processing in pinealocytes involves reciprocal changes in the

alpha1-adrenergic component of the cyclic GMP response and the beta-adrenergic component of the cyclic AMP response. J. Neurochem., 4, 678-686.

Vanecek, J., Pavlik, A. and Illnerova, H. (1987). Hypothalamic melatonin receptor sites revealed by autoradiography. Brain Res., 435, 359-362.

Vaughan, M.K., Vaughan, G.M., Reiter, R.J. and Benson, B. (1972). Effect of melatonin and other pineal indoles on adrenal enlargement produced in male and female mice by pinealectomy, unilateral adrenalectomy, castraton and cold stress. Neuroendocrinology, 10, 139-154.

Vetulani, J., Bednarczyk, B., Reichenberg, K. and Rokosz, A. (1980). Headtwitches induced by LSD and quipazine: Similarities and differences. Neuropharmcology, 19, 155-158.

Vollrath, L., Semm, P. and Gammel, G. (1981). Sleep induction by intranasal application of melatonin. In: Melatonin: Current Status and Perspectives. Eds. Birau, N. and Schloot, W., pp. 327-329. Oxford: Pergamon Press.

Vollrath, L., Schultz, R.L. and McMillan, P.J. (1983). "Synaptic" ribbons and spherules of the guinea pig pineal gland: Inverse day/night differences in number. Am. J. Anat., 168, 67-74.

Vollrath, L. (1984). Functional anatomy of the human pineal gland. In: The Pineal Gland. Ed. Reiter, R. J., pp. 285-322. New York: Raven Press.

Voisin, P., Namboodiri, M.A.A. and Klein, D.C. (1984). Arylamine N-acetyltransferase and arylalkylamine N-acetyltransferase in the mammalian pineal gland. J. Biol. Chem., 259, 10913-10918.

Waeber, C., Schoeffter, P., Palacios, J.M. and Hoyer, D. (1988). Molecular pharmacology of 5-HT1D recognition sites: Radioligand binding studies in human, pig and calf membranes. Naunyn-Schmiedeberg's Arch. Pharmacol., 337, 595-601.

Waldhauser, F., Lynch, H.J. and Wurtman, R.J. (1984a). Melatonin in human body fluids: Clinical significance. In: The Pineal Gland. Ed. Reiter, R.J., pp 345-372. New York: Raven Press.

Waldhauser, F., Leiberman, H.J., Frisch, H., Herkner, K. and Wurtman, R.J. (1984b). Increase of human serum prolactin levels after melatonin administration. EPSG-Newsletter Supplement, 5, 47.

Waldhauser, F., Saletu, B. and Trinchard-Lugan, I. (1990). Sleep laboratory investigations on hypnotic properties of melatonin. Psychopharmacology, 100, 222-226.

Wang, S.S.H., Mathis, C.A. and Peroutka, S.J. (1988). R(-)-2,5-dimethoxy-4-^{77}bromoamphetamine (^{77}Br-R(-)DOB): A novel radioligand which labels a 5-HT binding site subtype. Psychopharmacology, 94, 431-432.

Wang, S.S.H., Mathis, C.A. and Peroutka, S.J. (1988). *Erratum.* Psychopharmacology, 96, 430.

Wansley, R.A. and Holloway, F.A. (1976). Oscillations in retention performance after passive avoidance training. Learn Motiv., 1, 296-302.

Warbritton, III J.D., Stewart, R.M. and Baldessarini, R.J. (1978). Decreased locomotor activity and attenuation of amphetamine hyperactivity with intraventricular infusion of serotonin in the rat. Brain Res., 143, 373-382.

Weaver, D.R., Keohma, J. and Reppert, S.M. (1987). Maternal fetal communication of circadian phase in a precocious rodent the spiny mouse. Am. J. Physiol., 253, E401-E404.

Weaver, D.R., Namboodiri, M.A.A. and Reppert, S.M. (1988a). Iodinated melatonin mimics melatonin action and reveals discrete binding sites in fetal brain. FEBS. Lett., 228, 123-127.

Weaver, D.R., Rivkees, S.A. and Reppert, S.M. (1988b). Autoradiographic localization of iodomelatonin binding sites in the hypothalamus of three rodent species. Endocr. Soc. Abstr., 177, 265.

Wever, R.A. (1986). Characteristics of circadian rhythms in human functions. In: Melatonin in humans, Proceedings of the first international conference of melatonin in humans. Eds. Wurtman, R.J. and Waldhauser, F., pp. 291-352. Vienna, Austria.

Weichmann, A.F. (1986). Melatonin: parallels in pineal gland and retina. Experimental Eye Research, 42, 507-527.

Weiss, S., Sebben, M., Kemp, D.E. and Bockaert, J. (1986). Serotonin 5-HT$_1$ receptors mediate inhibtion of cyclic AMP production in neurons. Eur. J. Pharmacol., 120, 227-230.

Weissman, A. (1976). The discriminability of aspirin in arthritic and nonarthritic rats. Pharmacol. Biochem. Behav., 5, 583-586.

White, F.J. and Appel, J.B. (1982). Training dose as a factor in LSD-saline discrimination. Psychopharmacology, 76, 20-25.

White, H.L. and Wu, J.C. (1975). Multiple binding sites of human brain monoamine oxidase as indicated by substrate competition. J. Neurochem., 25, 21-26.

Wilkinson, M., Arendt, J., Bradtke, J. and De Ziegler, D. (1977). Determination of a dark-induced increase of pineal N-acetyl-transferase activity and simultaneous radioimmunoassay of melatonin in pineal, serum and pituitary tissue of the male rat. J. Endocr., 72, 243-244.

Williams, L.M. and Morgan, P.J. (1988). Demonstration of melatonin-binding sites of the pars tuberalis of the rat. J. Endocr., 119, R1-R3.

Wing, L.L., Tapson, G. S. and Geyer, M.A. (1990). 5-HT$_2$ mediation of

cute behavioural effects of hallucinogens in rats. Psychopharmacology, 100, 417-425.

Wirz-Justice, A. (1987). Circadian rhythms in mammalian neurotransmitter receptors. Prog. Neurobiol., 29, 219-259.

Wirz-Justice, A., Kraeuchi, K., Morimasa, T., Willener, R. and Feer, H. (1983). Circadian rhythm of [^{3}H]-imipramine binding in the rat suprachiasmatic nuclei. Eur. J. Pharmacol., 87, 331-333.

Wong, R. and Whiteside, C.B.C. (1968). The effect of melatonin on the wheel-running activity of rats deprived of food. J. Endocr., 40, 383-384.

Wurtman, R.J. and Axelrod, J. (1965). The pineal gland. Sci. Am., 213, 50-60.

Wurtman, R.J. and Anton-Tay, F. (1969). The mammalian pineal as a neuroendocrine transducer. Recent Prog. Horm. Res., 25, 493-514.

Wurtman, R.J., Axelrod, J.and Phillips, L.S. (1963). Melatonin synthesis in the pineal gland: Control by light. Science., 142, 1071-1073.

Wurtman, R.J., Axelrod, J. and Fischer, J.E. (1964a). Melatonin synthesis in the pineal gland: Effect of light mediated by the sympathetic nervous system. Science., 143, 1329-1330.

Wurtman, R.J., Axelrod, J. and Potter, L.T. (1964b). The uptake of [^{3}H]-melatonin in endocrine and nervous tissues and the effects of constant light exposure. J. Pharmacol. Exp. Ther., 143, 314-319.

Wurtman, R.J., Axelrod, J. and Kelly, D.E. (1968). The Pineal. New York: Academic Press.

Wurzburger, R.J., Kawashima, K., Miller, R.L. and Spector, S. (1976). Determination of rat pineal gland melatonin content by radioimmunoassay. Life Sci., 18, 867-878.

Yagaloff, K. A. and Hartig, P.R. (1985). ^{125}I-Lysergic acid diethylamide binds to a novel serotonergic site on rat choroid plexus epithelial cells. J. Neurosci., 5, 3178-3183.

Yap, C.Y. and Taylor, D.A. (1983). Involvement of 5-HT$_2$ receptors in the wet-dog shake behaviour induced by 5-hydroxytryptophan in the rat. Neuropharmacology, 22, 801-804.

Youdim, M.B.H. and Findberg, J.P.M. (1983). Monoamine oxidase inhibitor antidepressants. In: Psychopharmacology I. Part I, Preclinical Psychopharmacology. Eds. Graham-Smith, D.G. and Cowen, P.J., pp. 38-70. Amsterdam: Excerpta Medica

Young, R. and Dewey, W.L. (1982). Differentiation of the behavioural responses produced by barbiturates and benzodiazepines by the benzodiazepine antagonist Ro 15-1788. In: Drug Discrimination: Applications in CNS Pharmacology. Eds. Colpaert, F.C. and Slangen, J.L., pp. 235-240. Amsterdam: Elsevier.

Young, S.N. and Anderson, G.M. (1982). Factors influencing melatonin, 5-hydroxytryptophan, 5-hydroxyindoleacetic acid 5-hydroxytryptamine and tryptophan in rat pineal gland. Neuroendocrinology, 35, 464-468.

Yuwiler, A. (1983a). Vasoactive intestinal peptide stimulation of serotonin N-acetyltransferase activity: general characteristics. J. Neurochem., 41, 146-153.

Yuwiler, A. (1983b). Light and agonists alter pineal N-acetyltransferase induction by vasoactive intestinal polypeptide. Science, 220, 1082-1083.

Zatz, M. (1985a). Phorbol esters mimic alpha$_1$-adrenoceptor potentiation of serotonin N-acetyltransferase induction in the rat pineal. J. Neurochem., 45, 637-639.

Zatz, M. (1985b). Denervation supersensitivity of the rat pineal to norepinephrine-stimulated [^{3}H]-inositide turnover revealed by lithium and a convenient procedure. J. Neurochem., 45, 95-100.

Zatz, M. and Romero, J.A. (1978). Effects of calcium-free medium on the incubation of serotonin N-acetyltransferase in the rat pineal. Biochem. Pharmacol., 27, 2549-2553.

Zisapel, N. (1988a). *Erratum.* J. Endocr., 118, 168.

Zisapel, N. (1988b). Melatonin receptors revisited. J. Neural. Transm., 73, 1-5.

Zisapel, N. and Laudon, M. (1987). A novel melatonin antagonist affects melatonin-mediated processes *in vitro* and *in vivo*. Eur. J. Pharmacol., 136, 259-260.

Zisapel, N., Egozi, V. and Laudon, M. (1985). Circadian variations in the inhibition of dopamine release from adult and newborn rat hypothalamus by melatonin. Neuroendocrinology., 40, 102-108.

Zisapel, N., Nir, J. and Laudon, M. (1988). Circadian variations in melatonin-binding sites in discrete areas of the male rat brain. FEBS. Lett., 232, 172-176.

Zucker, I. (1971). Light-dark rhythms in rat eating and drinking behaviour. Physiol. Behav., 6, 115-126.

APPENDIX

APPENDIX

The Skinner boxes used in chapters 4 and 6 enabled a certain degree of automation in the running of drug discrimination experiments and in the collection and analysis of the data generated. However, they first had to be interfaced with a BBC Master computer and the appropriate software written. This was achieved using the SPIDER INTERFACE manufactured by Paul Fray Ltd. which expands the computer language, BBC Basic, with additional keywords recognizable only by SPIDER. The following pages lists the programs written by the author of this thesis. The first program was used for training animals to press the appropriate lever and the second for controlling the Skinner boxes during experiments and recording the appropriate responses. The final program listed was used for analysing the data and obtaining a printout of a particular training or experimental session (an example of an experimental session printout and its corresponding cumulative response graph is also shown).

LISTING OF SKINNER BOX TRAINING PROGRAM

```
 10REM ********PLOT-BOX PROGRAM*********
 20REM ********BY DM PACHE, 1988********
 30MODE128
 40:
 50REM ****CONSTANTS****
 60:
 70a1%=0
 80a2%=0
 90C%=1:D%=1
100E%=0:G%=0:F%=0:H%=0
110I%=0:K%=0:J%=0:L%=0
120M%=0:N%=0:O%=0:P%=0
130S%=0:T%=0:U%=0:V%=0
140Q%=0:R%=0
150a%=7
160b%=0
170c%=0
180d%=0
190e%=1
200f=0
210g=0
220:
230REM DEFINE ARRAYS
240DIMt_one(1750):i%=0
250DIMt_two(1750):j%=0
260:
270REM DISPLAY
280PRINT TAB(4,4)"This is the advanced rat-training program, designed to "
290PRINT TAB(4,6)"increase the number of times a rat presses a lever for a "
300PRINT TAB(4,8)"food 'reward'."
310PRINT TAB(4,10)"The desired FR value MUST be attained gradually. Increases"
320PRINT TAB(4,12)"in the FR value should be made in steps of 2 or 3 each "
330PRINT TAB(4,14)"week until FR10 is achieved if a two session per day "
340PRINT TAB(4,16)"routine is used. From then on it is possible to use steps"
350PRINT TAB(4,18)"of 5. Each session is 15 mins long."
360PRINT TAB(40,20)"By D.M. Pache, 1988"
370REPEAT: PRINT TAB(10,30)"PRESS 'C' TO CONTINUE ";:S$=GET$:UNTIL S$="C"
380MODE128
390PRINT TAB(4,0)"Take care to input the information correctly"
400REM *****PROG-PROPER*****
410:
420INPUT TAB(4,2)"ENTER TODAY'S DATE......"E$
430PRINT TAB(4,4)"ENTER the conditions under which the rat is being subjected,"
435INPUT TAB(4,6)"eg LIGHT/DARK OR TRAINING ";H$
440INPUT TAB(4,8)"ENTER time of start (1st injection time) ";TM$
445INPUT TAB(4,10)"ENTER time of food withdrawal ";FW$
450INPUT TAB(4,12)"ENTER details of any drug/dose used"G$
460INPUT TAB(4,14)"ENTER the length of the session (usually 15 mins)"h%
470INPUT TAB(4,16)"ENTER the filename you wish to save the data under"F$
480INPUT TAB(4,18)"ASSIGN FR VALUE...."n%
490INPUT TAB(4,20)"HOW LONG CAN THE ANIMALS FEED FOR...."o%
500INPUT TAB(4,22)"First Rat No....."p%
510INPUT TAB(4,24)"Second Rat No....."q%
515INPUT TAB(4,26)"ENTER CHOSEN RAT (A or B)";RAT$
520:
530REM ****ASSIGN SWITCH CONSTANTS FOR 'CORRECT' AND 'WRONG' LEVERS****
540:
550REPEAT:PRINT "ASSIGN REWARD LEVER FOR BOX 1....":A$=GET$:UNTIL A$="L" OR A$="R"
560IF A$="L" THEN A$="LEFT":c1%=1: C$="RIGHT": w1%=0
562PRINT TAB(35,27)A$:ELSE c1%=0:A$="RIGHT":C$="LEFT":w1%=1
564PRINT TAB(35,27)A$
570REPEAT:PRINT "ASSIGN REWARD LEVER FOR BOX 2...."
572B$=GET$:UNTIL B$="L" OR B$="R"
574IF B$="L" THEN B$="LEFT": c2%=4: D$="RIGHT": w2%=7
```

```
576PRINT TAB(35,28)B$:ELSE c2%=7:B$="RIGHT":D$="LEFT":w2%=4
578PRINT TAB(35,28)B$
580IF B$="L" THEN B$="LEFT": c2%=4: D$="RIGHT": w2%=7
582PRINT TAB(35,28)B$:ELSE c2%=7:B$="RIGHT":D$="LEFT":w2%=4
583PRINT TAB(35,28)B$
585REPEAT: PRINT TAB(10,30)"CHECK INFO. PRESS 'C' TO CONTINUE ";
587S$=GET$:UNTIL S$="C"
590:
600REM ***** ASSIGN SWITCH CONSTANTS FOR BOX 1 (LEFT)******
610:
620r%=8
630t%=2
640v%=3
650:
660REM ****ASSIGN SWITCH CONSTANTS FOR BOX 2 (RIGHT)*****
670:
680s%=9
690u%=6
700w%=5
710:
720:
730REM ****INITIALIZE THE SYSTEM****
740KILL ALL
750GOVN SWITCH r% TO s%
760FREE SWITCH 0 TO 7
770:
780:
790REM ****SCREEN DISPLAY****
800:
810CLS
820:
830PRINT TAB(2,3)"Date..."E$
840PRINT TAB(24,3)"Filename..."F$
845PRINT TAB(50,3)"Start time..."TM$
850PRINT TAB(2,4)"Drug..."G$
860PRINT TAB(24,4)"Conditions..."H$
865PRINT TAB(50,4)"CHOSEN RAT..."RAT$
870PRINT TAB(30,5)"BOX No. A"
880PRINT TAB(10,7)"Rat No. ";p%
890PRINT TAB(30,7)"FR Value ";n%
900PRINT TAB(50,6)"Reward lever "A$
910PRINT TAB(50,7)"Wrong lever  "C$
920PRINT TAB(30,19)"BOX No. B"
930PRINT TAB(10,21)"Rat No. ";q%
940PRINT TAB(30,21)"FR Value ";n%
950PRINT TAB(50,20)"Reward lever "B$
960PRINT TAB(50,21)"Wrong lever  "D$
970REPEAT:PRINT TAB(0,0)"Press 'C' to continue, then IMMEDIATELY
975PRINT TAB(0,0)"after press F9 to start the timer ";
977CON$=GET$:UNTIL CON$="C"
980:
990:
1000REM ****HERE COMES THE WORKING PART OF THE PROGRAM****
1010:
1020¦(SWITCH c1%, ON) PROCreward_1
1030¦(fc1%) PROCkey(c1%)
1040¦(SWITCH c2%, ON) PROCreward_2
1050¦(fc2%) PROCkey(c2%)
1060¦(SWITCH w1%, ON) PROCwrong_1
1070¦(fw1%) PROCkey(w1%)
1080¦(SWITCH w2%, ON) PROCwrong_2
1090¦(fw2%) PROCkey(w2%)
1100¦(f2) PROCdip_up_1
1110¦(f3) PROCdip_up_2
1120¦(f9) PROCtimer
1130:
1140REM ****AWAIT****
```

```
1150:
1160WAIT
1170:
1180:
1190END
1200:
1210REM ***** HERE ARE THE PROCEDURES *****
1220:
1230DEFPROCtimer
1240x%=0:TIME=0
1250PRINT TAB(0,0)"EXPT IN PROGRESS........                                    "
1260¦(TIMER a%,h%*100*60) PROCfinish_1
1280ENDPROC
1290:
1300DEFPROCend
1310PRINT TAB(0,0)"TIME="h%" MINS, EXPT COMPLETE....REMOVE RATS"
1320PROCoverture
1330ENDPROC
1340:
1350DEFPROCkey(A%)
1360^SWITCH A%
1370ENDPROC
1380:
1390DEFPROCreward_1
1400b%=b%+1
1410E%=E%+1
1420PRINT TAB(2,10) "REWARD-LEVER (";A$") COUNT........";E%
1430t_one(i%)=TIME/100
1435PRINT TAB(2,11)"TIME AT LAST R LEVER PRESS = ";t_one(i%):i%=i%+1
1440IF E%=n% AND C% THEN PROCcal_FR_1
1450PROCtotalcounts_1
1460IF b%=n% THEN KILL SWITCH c1%:KILL SWITCH w1%
1470IF b%=n% THEN PROCdip_up_1
1480ENDPROC
1490:
1500:
1510DEFPROCreward_2
1520c%=c%+1
1530F%=1+F%
1540PRINT TAB(2,23) "REWARD-LEVER (";B$") COUNT........";F%
1550t_two(j%)=TIME/100
1555PRINT TAB(2,24)"TIME AT LAST R LEVER PRESS = ";t_two(j%):j%=j%+1
1560IF F%=n% AND D% THEN PROCcal_FR_2
1570PROCtotalcounts_2
1580IF c%=n% THEN KILL SWITCH c2%:KILL SWITCH w2%
1590IF c%=n% THEN PROCdip_up_2
1600ENDPROC
1610:
1620DEFPROCwrong_1
1630I%=I%+1
1640PRINT TAB(43,10) "WRONG-LEVER ("C$") COUNT.........";I%
1650PROCtotalcounts_1
1660ENDPROC
1670:
1680DEFPROCwrong_2
1690J%=J%+1
1700PRINT TAB(43,23) "WRONG-LEVER (";D$") COUNT.........";J%
1710PROCtotalcounts_2
1720ENDPROC
1730:
1740DEFPROCcal_FR_1
1750C%=0
1760G%=E%
1770K%=I%
1780M%=E%/(E%+I%)*100
1790N%=100-M%
```

```
1800f1%=TIME
1810IF a1%=0 THEN PROCtype_1
1820ENDPROC
1830:
1840DEFPROCcal_FR_2
1850D%=0
1860H%=F%
1870 L%=J%
18800%=F%/(F%+J%)*100
1890P%=100-0%
1900f2%=TIME
1910IF a2%=0 THEN PROCtype_2
1920ENDPROC
1930:
1940DEFPROCtype_1
1950PRINT TAB(43,11) "WRONG lever (";C$") count at FRF = ";I%
1960PRINT TAB(2,12) "FRF value for REWARD lever......";M%
1970PRINT TAB(43,12) "FRF value for WRONG lever.....";N%
1980PRINT TAB(2,13) "Time at FRF value =      ";(f1%-x%)/100" secs":f=(f1%-x%)/100
1990a1%=1
2000ENDPROC
2010:
2020DEFPROCtype_2
2030PRINT TAB(43,24) "WRONG lever (";D$") count at FRF = ";J%
2040PRINT TAB(2,25) "FRF value for REWARD lever......";0%
2050PRINT TAB(43,25) "FRF value for WRONG lever.....";P%
2060PRINT TAB(2,26) "Time at FRF value =      ";(f2%-x%)/100" secs":g=(f2%-x%)/100
2070a2%=1
2080ENDPROC
2090:
2100DEFPROCoverture
2110SOUND 10,-10,100,5
2120SOUND 10,1,10,1
2130SOUND 10,-10,100,5
2140SOUND 10,1,10,1
2150SOUND 10,-10,100,5
2160SOUND 10,1,10,1
2170SOUND 10,-12,85,20
2180PROCsave_data
2190ENDPROC
2200:
2210DEFPROCtotalcounts_1
2220PRINT TAB(55,16) "TOTAL COUNTS = ";E%+I%
2230ENDPROC
2240:
2250DEFPROCtotalcounts_2
2260PRINT TAB(55,29) "TOTAL COUNTS = ";F%+J%
2270ENDPROC
2280:
2290REM ****HERE ARE THE PROCEDURES THAT WORK THE DIPPERS****
2300:
2310REM ****FIRST BOX 1****
2320:
2330DEFPROCdip_up_1
2340b%=0
2350SWITCH ON r%
2360¦(SWITCH t%, ON) PROCstop_1
2370ENDPROC
2380:
2390DEFPROCstop_1
2400SWITCH OFF r%
2410PROCfeed_time_1
2420ENDPROC
2430:
2440DEFPROCfeed_time_1
```

```
3085 IF RS$="Y" THEN PROCdisc ELSE 3100
3100REPEAT:PRINT TAB(0,2)"DO YOU WISH TO PLOT THE DATA....."
3105UNTIL GS$="Y" OR GS$="N":PRINT TAB(35,2)GS$
3110 IF GS$="Y" THEN PROCplot ELSE 1190
3120ENDPROC
3130:
3140DEFPROCdisc
3150X=OPENOUTF$
3160PTR£X=EXT£X
3170PRINT£X,E$,H$,G$,F$,h%,TM$,FW$,RAT$
3180PRINT£X,p%,q%,n%,A$,B$,C$,D$
3190PRINT£X,G%,K%,H%,L%,E%,I%,F%,J%
3200PRINT£X,M%,N%,O%,P%
3210PRINT£X,f,g
3220PRINT£X,S%,T%,U%,V%
3230save_1%=0
3240REPEAT
3250PRINT£X,t_one(save_1%):save_1%=save_1%+1
3260UNTIL t_one(save_1%)=0 OR t_one(1750)
3270save_2%=0
3280REPEAT
3290PRINT£X,t_two(save_2%):save_2%=save_2%+1
3300UNTIL t_two(save_2%)=0 OR t_two(1750)
3310CLOSE£X
3320ENDPROC
3330:
3340DEFPROCplot
3350CLS
3360REM CREATE GRAPHICS WINDOW
3370VDU 24,30;20;1279;1023
3380REM DRAW AXIS
3390MOVE 0,0
3400PLOT 0,220,170
3410PLOT 1,0,800
3420PLOT 0,0,-800
3430PLOT 1,900,0
3440PLOT 0,-900,0
3450MOVE 220,170
3460FOR x_plot%=0 TO 5:MOVE 900*x_plot%/5+220,170:PLOT 1,0,-20:PLOT 0,0,20:NEXT
3470MOVE 220,130
3480FOR y_plot%=0 TO 5
3490MOVE 220,800*y_plot%/5+170
3500PLOT 1,-20,0
3510PLOT 0,20,0
3520NEXT
3530:
3540REM X-CO-ORDS
3550MOVE 220,170
3560VDU 5
3570FOR q%=0 TO 5
3580MOVE 900*q%/5+220,130
3590PRINT;q%*h%*60/5
3600NEXT
3610MOVE 800+370,130:PRINT;"secs"
3620FOR min%=0 TO 5
3630MOVE 900*min%/5+220,70
3640PRINT;min%*h%/5
3650NEXT
3660MOVE 800+370,70:PRINT;"mins"
3670:
3680REM Y-CO-ORDS
3690MOVE 220,170
3700FOR p%=0 TO 5
3710MOVE 120,800*p%/5+170
3720PRINT;p%*2000/5
```

```
3730NEXT
3740MOVE 80,1000:PRINT;"C"
3750MOVE 80,960:PRINT;"O"
3760MOVE 80,920:PRINT;"U"
3770MOVE 80,880:PRINT;"N"
3780MOVE 80,840:PRINT;"T"
3790MOVE 80,800:PRINT;"S"
3800:
3810t_1%=0
3820MOVE 220,170
3830REPEAT
3840DRAW 220+t_one(t_1%)*(900/(h%*60)),t_1%*800/2000+170
3850t_1%=t_1%+1
3860UNTIL t_one(t_1%)=0 OR t_one(1750)
3870PRINT; "   RAT A"
3880MOVE 0,0
3890:
3900t_2%=0
3910MOVE 220,170
3920REPEAT
3930DRAW 220+t_two(t_2%)*(900/(h%*60)),t_2%*800/2000+170
3940t_2%=t_2%+1
3950UNTIL t_two(t_2%)=0 OR t_two(1750)
3960PRINT; "   RAT B"
3970MOVE 0,0
3980ENDPROC
3990:
4000END
```

LISTING OF SKINNER BOX EXPERIMENTAL PROGRAM

```
 10REM ********PLOT-BOX PROGRAM EXPERIMENT**********
 20REM ********BY DM PACHE, 1988********
 30MODE128
 40:
 50REM ****CONSTANTS****
 60:
 70a%=0
 80b%=0
 90nd_l1%=1:nd_l2%=1:nd_r1%=1:nd_r2%=1
100:
110REM ***LEVER COUNT CONSTANTS***
12011%=0:12%=0:r1%=0:r2%=0
130:
140FRFa_1%=0:FRFb_1%=0:FRFa_2%=0:FRFb_2%=0
150FRF_t_cl_1%=0:FRF_t_cr_1%=0:FRF_t_cl_2%=0:FRF_t_cr_2%=0
160totalcounts_1%=0:totalcounts_2%=0
170countdown%=7
180lc1%=0
190rc1%=0
200lc2%=0
210rc1%=0
220 FRcount_l1%=0:FRcount_r1%=0:FRcount_l2%=0:FRcount_r2%=0
225 FRrej_1%=0:FRrej_2%=0
230:
240REM ***TIME CONSTANTS***
250time_1%=0
260time_2%=1
270time_1=0
280time_2=0
290:
300REM ***STRINGS***
310chosen_1$="NONE":rej_lev1$="NONE"
320chosen_2$="NONE":rej_lev2$="NONE"
330:
340REM ***SWITCH CONSTANTS***
350left_1%=1
360right_1%=0
370left_2%=4
380right_2%=7
390:
400c_left_1%=0
410c_right_1%=0
420c_left_2%=0
430c_right_2%=0
440c_cho_1%=0
450c_rej_1%=0
460c_rej_2%=0
470c_cho_2%=0
480:
490REM DEFINE ARRAYS
500DIMt_one(1200):dim_a%=0
510DIMt_two(1200):dim_b%=0
520:
530REM DISPLAY
540PRINT TAB(4,4)"This is the rat EXPERIMENT program, designed to "
550PRINT TAB(4,6)"reward an animal if it presses either lever a "
560PRINT TAB(4,8)"designated number of times. This becomes the CHOSEN "
570PRINT TAB(4,10)"lever, while the other assumes the REJECT identity. "
580PRINT TAB(4,12)"Each session is 5 mins long."
590PRINT TAB(40,20)"By D.M. Pache, 1988"
600REPEAT: PRINT TAB(10,30)"PRESS 'C' TO CONTINUE ";:S$=GET$:UNTIL S$="C"
610MODE128
620PRINT TAB(4,4)"Take care to input the information correctly"
630REM *****PROG-PROPER*****
```

```
640:
650INPUT TAB(4,6)"ENTER TODAY'S DATE......"D$
660PRINT TAB(4,7)"ENTER the conditions under which the rat is being subjected,"
670INPUT TAB(4,8)"eg LIGHT/DARK/MD/ML/EOD/EOL";C$
680INPUT TAB(4,9)"TIME OF EXPT (IN 24hr notation, eg 18-30)";TM$
685INPUT TAB(4,10)"ENTER time of food withdrawal";FW$
690INPUT TAB(4,11)"ENTER details of any drug/dose used";DD$
700INPUT TAB(4,12)"ENTER details of PRE-TREATMENT TIME";T$
710INPUT TAB(4,13)"ENTER the length of the session (usually 15 mins)";DT%
720INPUT TAB(4,14)"ENTER the filname you wish to save the data under";filename$
730INPUT TAB(4,16)"ASSIGN FR VALUE...."FRa%
740INPUT TAB(4,18)"HOW LONG CAN THE ANIMALS FEED FOR...."feed_time%
750INPUT TAB(4,20)"First Rat No....."RAT_1%
760REPEAT:PRINT TAB(4,22)"Enter LIGHT lever for first rat...":L1$=GET$
765UNTIL L1$="L" OR L1$="R"
770IF L1$="L" THEN L1$="LEFT":D1$="RIGHT"
775PRINT TAB(40,22)L1$ ELSE L1$="RIGHT":D1$="LEFT":PRINT TAB(40,22)L1$
780INPUT TAB(4,24)"Second Rat No....."RAT_2%
790REPEAT:PRINT TAB(4,26)"Enter LIGHT lever for SECOND rat...":L2$=GET$
795UNTIL L2$="L" OR L2$="R"
800IF L2$="L" THEN L2$="LEFT":D2$="RIGHT"
805PRINT TAB(40,26)L2$: ELSE L2$="RIGHT":D2$="LEFT":PRINT TAB(40,26)L2$
810REPEAT: PRINT TAB(10,30)"CHECK INFO. PRESS 'C' TO CONTINUE ";:S$=GET$
815UNTIL S$="C"
820:
830REM ***** ASSIGN SWITCH CONSTANTS FOR BOX 1 (LEFT)******
840:
850motor_1%=8
860dipper_up_1%=2
870dipper_down_1%=3
880:
890REM ****ASSIGN SWITCH CONSTANTS FOR BOX 2 (RIGHT)*****
900:
910motor_2%=9
920dipper_up_2%=6
930dipper_down_2%=5
940:
950:
960REM ****INITIALIZE THE SYSTEM****
970KILL ALL
980GOVN SWITCH motor_1% TO motor_2%
990FREE SWITCH 0 TO 7
1000:
1010:
1020REM ****SCREEN DISPLAY****
1030:
1040CLS
1050:
1060PRINT TAB(2,3)"Date..."D$
1070PRINT TAB(30,3)"Filename..."filename$
1080PRINT TAB(2,4)"Drug..."DD$
1090PRINT TAB(30,4)"Conditions..."C$
1100PRINT TAB(60,3)"FR Value = ";FRa%
1110PRINT TAB(60,4)"Pre-T Time = ";T$
1120PRINT TAB(30,5)"BOX No. 1"
1130PRINT TAB(10,7)"Rat No. ";RAT_1%
1140PRINT TAB(60,7)"LIGHT lever = ";L1$
1150PRINT TAB(30,7)"CHOSEN lever = ";chosen_1$
1160PRINT TAB(30,8)"REJECT lever = ";rej_lev1$
1170PRINT TAB(30,19)"BOX No. 2"
1180PRINT TAB(10,21)"Rat No. ";RAT_2%
1190PRINT TAB(60,21)"LIGHT lever = "L2$
1200PRINT TAB(30,21)"CHOSEN lever = ";chosen_2$
1210PRINT TAB(30,22)"REJECT lever = ";rej_lev2$
1220REPEAT
1222PRINT TAB(0,0)"Press 'C' to continue, then IMMEDIATELY after press F9 to start the timer "
1224CON$=GET$:UNTIL CON$="C"
```

```
1230:
1240:
1250REM ****HERE COMES THE WORKING PART OF THE PROGRAM****
1260:
1270¦(SWITCH left_1%, ON) PROCl_1
1280¦(fleft_1%) PROCkey(left_1%)
1290¦(SWITCH left_2%, ON) PROCl_2
1300¦(fleft_2%) PROCkey(left_2%)
1310¦(SWITCH right_1%, ON) PROCr_1
1320¦(fright_1%) PROCkey(right_1%)
1330¦(SWITCH right_2%, ON) PROCr_2
1340¦(fright_2%) PROCkey(right_2%)
1350¦(f2) PROCdip_up_1
1360¦(f3) PROCdip_up_2
1370¦(f9) PROCtimer
1380:
1390REM ****AWAIT****
1400:
1410WAIT
1420:
1430:
1440END
1450:
1460REM ***** HERE ARE THE PROCEDURES *****
1470:
1480DEFPROCtimer
1490start_time%=0:TIME=0
1500PRINT TAB(0,0)"EXPT IN PROGRESS........                                    "
1510¦(TIMER countdown%,DT%*100*60) PROCfinish_1
1520ENDPROC
1530:
1540DEFPROCend
1550PRINT TAB(0,0)"TIME="DT%" MINS, EXPT COMPLETE....REMOVE RATS"
1560PROCoverture
1570ENDPROC
1580:
1590DEFPROCkey(A%)
1600^SWITCH A%
1610ENDPROC
1620:
1630DEFPROCl_1
1640lc1%=1+lc1%
1650c_left_1%=c_left_1%+1
1660PRINT TAB(1,10) "LEFT-LEVER COUNT........";c_left_1%
1670IF chosen_1$="LEFT" THEN t_one(dim_a%)=TIME/100
1675PRINT TAB(1,11)"TIME AT LAST R LEVER PRESS = ";t_one(dim_a%):dim_a%=dim_a%+1
1680IF c_left_1%=FRa% AND c_right_1%<FRa% AND nd_l1% THEN PROCass_L1
1690PROCtotalcounts_1
1700IF lc1%=FRa% AND l1%=1 THEN KILL SWITCH left_1%:KILL SWITCH right_1%
1710IF lc1%=FRa% AND l1%=1 THEN PROCdip_up_1
1720ENDPROC
1730:
1740DEFPROCass_L1
1750chosen_1%=1:rej_1%=0:chosen_1$="LEFT":rej_lev1$="RIGHT"
1760c_cho_1%=c_left_1%
1770c_rej_1%=c_right_1%
1780l1%=1
1790PRINT TAB(30,7)"CHOSEN LEVER = ";chosen_1$
1800PRINT TAB(30,8)"REJECT LEVER = ";rej_lev1$
1810IF chosen_1$=L1$ THEN PRINT TAB(60,8)"LIGHT lever chosen"
1815ELSE PRINT TAB(60,8)"DARK lever chosen"
1820PROCcal_FR_1
1830ENDPROC
```

```
1840:
1850DEFPROCl_2
1860lc2%=lc2%+1
1870c_left_2%=c_left_2%+1
1880PRINT TAB(1,24) "LEFT LEVER COUNT........";c_left_2%
1890IF chosen_2$="LEFT" THEN t_two(dim_b%)=TIME/100
1895PRINT TAB(1,25)"TIME AT LAST R LEVER PRESS = ";t_two(dim_b%):dim_b%=dim_b%+1
1900IF c_left_2%=FRa% AND c_right_2%<FRa% AND nd_12% THEN PROCass_L2
1910PROCtotalcounts_2
1920IF lc2%=FRa% AND 12%=1 THEN KILL SWITCH left_2%:KILL SWITCH right_2%
1930IF lc2%=FRa% AND 12%=1 THEN PROCdip_up_2
1940ENDPROC
1950:
1960DEFPROCass_L2
1970chosen_2%=4:rej_2%=7:chosen_2$="LEFT":rej_lev2$="RIGHT"
1980c_cho_2%=c_left_2%
1990c_rej_2%=c_right_2%
200012%=1
2010PRINT TAB(30,21)"CHOSEN LEVER = ";chosen_2$
2020IF chosen_2$=L2$ THEN PRINT TAB(60,22)"LIGHT lever chosen"
2025ELSE PRINT TAB(60,22)"DARK lever chosen"
2030PRINT TAB(30,22)"REJECT LEVER = ";rej_lev2$
2040PROCcal_FR_2
2050ENDPROC
2060:
2070DEFPROCr_1
2080rc1%=rc1%+1
2090c_right_1%=c_right_1%+1
2100PRINT TAB(40,10) "RIGHT-LEVER COUNT.........";c_right_1%
2110IF chosen_1$="RIGHT" THEN t_one(dim_a%)=TIME/100
2115PRINT TAB(1,11)"TIME AT LAST R LEVER PRESS = ";t_one(dim_a%):dim_a%=dim_a%+1
2120IF c_right_1%=FRa% AND c_left_1%<FRa% AND nd_r1% THEN PROCass_R1
2130PROCtotalcounts_1
2140IF rc1%=FRa% AND r1%=1 THEN KILL SWITCH left_1%:KILL SWITCH right_1%
2150IF rc1%=FRa% AND r1%=1 THEN PROCdip_up_1
2160ENDPROC
2170:
2180DEFPROCass_R1
2190chosen_1%=0:rej_1%=1:chosen_1$="RIGHT":rej_lev1$="LEFT"
2200c_rej_1%=c_left_1%
2210c_cho_1%=c_right_1%
2220r1%=1
2230PRINT TAB(30,7)"CHOSEN LEVER = ";chosen_1$
2240IF chosen_1$=L1$ THEN PRINT TAB(60,8)"LIGHT lever chosen"
2245ELSE PRINT TAB(60,8)"DARK lever chosen"
2250PRINT TAB(30,8)"REJECT LEVER = ";rej_lev1$
2260PROCcal_FR_1
2270ENDPROC
2280:
2290DEFPROCr_2
2300rc2%=rc2%+1
2310c_right_2%=c_right_2%+1
2320PRINT TAB(40,24) "RIGHT-LEVER COUNT.........";c_right_2%
2330IF chosen_2$="RIGHT" THEN t_two(dim_b%)=TIME/100
2335PRINT TAB(1,25)"TIME AT LAST R LEVER PRESS = ";t_two(dim_b%):dim_b%=dim_b%+1
2340IF c_right_2%=FRa% AND c_left_2%<FRa% AND nd_r2% THEN PROCass_R2
2350PROCtotalcounts_2
2360IF rc2%=FRa% AND r2%=1 THEN KILL SWITCH left_2%:KILL SWITCH right_2%
2370IF rc2%=FRa% AND r2%=1 THEN PROCdip_up_2
2380ENDPROC
2390:
2400DEFPROCass_R2
2410chosen_2%=4:rej_1%=7:chosen_2$="RIGHT":rej_lev2$="LEFT"
2420c_rej_2%=c_left_2%
2430c_cho_2%=c_right_2%
```

```
2440r2%=1
2450PRINT TAB(30,21)"CHOSEN LEVER = ";chosen_2$
2460IF chosen_2$=L2$ THEN PRINT TAB(60,22)"LIGHT lever chosen"
2465ELSE PRINT TAB(60,22)"DARK lever chosen"
2470PRINT TAB(30,22)"REJECT LEVER = ";rej_lev2$
2480PROCcal_FR_2
2490ENDPROC
2500:
2510DEFPROCcal_FR_1
2520nd_l1%=0
2530nd_r1%=0
2540FRcount_l1%=c_left_1%
2550FRcount_r1%=c_right_1%
2560FRFa_1%=c_cho_1%/(c_cho_1%+c_rej_1%)*100
2570FRFb_1%=100-FRFa_1%
2580FR_time_1%=TIME
2590IF a%=0 THEN PROCtype_1
2600ENDPROC
2610:
2620DEFPROCcal_FR_2
2630nd_l2%=0
2640nd_r2%=0
2650FRcount_l2%=c_left_2%
2660FRcount_r2%=c_right_2%
2670FRFa_2%=c_cho_2%/(c_cho_2%+c_rej_2%)*100
2680FRFb_2%=100-FRFa_2%
2690FR_time_2%=TIME
2700IF b%=0 THEN PROCtype_2
2710ENDPROC
2720:
2730DEFPROCtype_1
2740FRrej_1%=c_rej_1%
2750PRINT TAB(40,11) "REJECTED lever (";rej_lev1$") count at FRF = ";FRrej_1%
2760PRINT TAB(1,12) "FRF value for CHOSEN lever...";FRFa_1%
2770PRINT TAB(40,12) "FRF value for REJECT lever...";FRFb_1%
2780PRINT TAB(1,13) "Time at FRF value = ";(FR_time_1%-start_time%)/100" secs"
2785time_1=(FR_time_1%-start_time%)/100
2790a%=1
2800ENDPROC
2810:
2820DEFPROCtype_2
2830FRrej_2%=c_rej_2%
2840PRINT TAB(40,25) "REJECTED lever (";rej_lev2$") count at FRF = ";FRrej_2%
2850PRINT TAB(1,26) "FRF value for REWARD lever...";FRFa_2%
2860PRINT TAB(40,26) "FRF value for REJECT lever...";FRFb_2%
2870PRINT TAB(1,27) "Time at FRF value = ";(FR_time_2%-start_time%)/100" secs"
2875time_2=(FR_time_2%-start_time%)/100
2880b%=1
2890ENDPROC
2900:
2910DEFPROCoverture
2920SOUND 10,-10,100,5
2930PROCsave_data
2940ENDPROC
2950:
2960DEFPROCtotalcounts_1
2970PRINT TAB(55,16) "TOTAL COUNTS = ";c_left_1%+c_right_1%
2980ENDPROC
2990:
3000DEFPROCtotalcounts_2
3010PRINT TAB(55,30) "TOTAL COUNTS = ";c_left_2%+c_right_2%
3020ENDPROC
3030:
3040REM ****HERE ARE THE PROCEDURES THAT WORK THE DIPPERS****
3050:
3060REM ****FIRST BOX 1****
```

```
3070:
3080DEFPROCdip_up_1
3090lc1%=0:rc1%=0
3100SWITCH ON motor_1%
3110¦(SWITCH dipper_up_1%, ON) PROCstop_1
3120ENDPROC
3130:
3140DEFPROCstop_1
3150SWITCH OFF motor_1%
3160PROCfeed_time_1
3170ENDPROC
3180:
3190DEFPROCfeed_time_1
3200¦(TIMER 0, feed_time%*100) PROCdip_down_1
3210ENDPROC
3220:
3230DEFPROCdip_down_1
3240SWITCH ON motor_1%
3250¦(SWITCH dipper_down_1%, ON) PROCno_feed_1
3260ENDPROC
3270:
3280DEFPROCno_feed_1
3290SWITCH OFF motor_1%
3300¦(SWITCH left_1%, ON) PROCl_1
3310¦(SWITCH right_1%, ON) PROCr_1
3320ENDPROC
3330:
3340REM ****BOX 2****
3350:
3360DEFPROCdip_up_2
3370lc2%=0:rc2%=0
3380SWITCH ON motor_2%
3390¦(SWITCH dipper_up_2%, ON) PROCstop_2
3400ENDPROC
3410:
3420DEFPROCstop_2
3430SWITCH OFF motor_2%
3440PROCfeed_time_2
3450ENDPROC
3460:
3470DEFPROCfeed_time_2
3480¦(TIMER 1, feed_time%*100) PROCdip_down_2
3490ENDPROC
3500:
3510DEFPROCdip_down_2
3520SWITCH ON motor_2%
3530¦(SWITCH dipper_down_2%, ON) PROCno_feed_2
3540ENDPROC
3550:
3560DEFPROCno_feed_2
3570SWITCH OFF motor_2%
3580¦(SWITCH left_2%, ON) PROCl_2
3590¦(SWITCH right_2%, ON) PROCr_2
3600ENDPROC
3610:
3620DEFPROCfinish_1
3630IF chosen_1$="LEFT" AND c_left_1%>FRa%-1 THEN c_cho_1%=c_left_1%:c_rej_1%=c_right_1%
3640IF chosen_1$="RIGHT" AND c_right_1%>FRa%-1 THEN c_cho_1%=c_right_1%:c_rej_1%=c_left_1%
3650IF c_left_1%<1 AND c_right_1%<1 THEN 3680
3660IF c_left_1%<1 AND c_right_1%>0 THEN FRF_t_cr_1%=c_right_1%/(c_left_1%+c_right_1%)*100
3665FRF_t_cl_1%=100-FRF_t_cr_1%
3670IF c_left_1%>0 THEN FRF_t_cl_1%=c_left_1%/(c_left_1%+c_right_1%)*100
3675FRF_t_cr_1%=100-FRF_t_cl_1%
3680PRINT TAB(1,15) "End FRF Value for LEFT lever = ";FRF_t_cl_1%
3690PRINT TAB(1,16) "End FRF Value for RIGHT lever = ";FRF_t_cr_1%
3700PROCfinish_2
3710ENDPROC
```

```
3730DEFPROCfinish_2
3740IF chosen_2$="LEFT" AND c_left_2%>FRa%-1 THEN c_cho_2%=c_left_2%:c_rej_2%=c_right_2%
3750IF chosen_2$="RIGHT" AND c_right_2%>FRa%-1 THEN c_cho_2%=c_right_2%:c_rej_2%=c_left_2%
3760IF c_left_2%<1 AND c_right_2%<1 THEN 3790
3770IF c_left_2%<1 AND c_right_2%>0 THEN FRF_t_cr_2%=c_right_2%/(c_left_2%+c_right_2%)*100
3775FRF_t_cl_2%=100-FRF_t_cr_2%
3780IF c_left_2%>0 THEN FRF_t_cl_2%=c_left_2%/(c_left_2%+c_right_2%)*100
3785FRF_t_cr_2%=100-FRF_t_cl_2%
3790PRINT TAB(1,29) "End FRF Value for LEFT lever = ";FRF_t_cl_2%
3800PRINT TAB(1,30) "End FRF Value for RIGHT lever = ";FRF_t_cr_2%
3810PROCend
3820ENDPROC
3830:
3840DEFPROCsave_data
3850KILL ALL
3860SWITCH OFF 8 TO 9
3870INPUT "DO YOU WISH TO SAVE THE DATA...(enter Y/N)...."RESPONSE$
3880IF RESPONSE$="Y" THEN PROCdisc
3890INPUT "DO YOU WISH TO PLOT THE DATA"GRAPH$
3900IF GRAPH$="Y" THEN PROCplot ELSE 1440
3910ENDPROC
3920:
3930DEFPROCdisc
3940CLS
3950X=OPENOUTfilename$
3960PTR£X=EXT£X
3970PRINT£X,D$,C$,DD$,filename$,DT%,TM$,FW$,T$,L1$,L2$
3980PRINT£X,RAT_1%,RAT_2%,FRa%,chosen_1$,chosen_2$,rej_lev1$,rej_lev2$
3990PRINT£X,FRcount_l1%,FRcount_r1%,FRcount_l2%,FRcount_r2%,c_left_1%
3992PRINT£X,c_right_1%,c_left_2%,c_right_2%,c_cho_1%,c_rej_1%,c_cho_2%
3994PRINT£X,c_cho_2%,c_rej_2%,FRrej_1%,FRrej_2%
4000PRINT£X,FRFa_1%,FRFb_1%,FRFa_2%,FRFb_2%
4010PRINT£X,time_1,time_2
4020PRINT£X,FRF_t_cl_1%,FRF_t_cr_1%,FRF_t_cl_2%,FRF_t_cr_2%
4030save_1%=0
4040REPEAT
4050PRINT£X,t_one(save_1%):save_1%=save_1%+1
4060PRINT TAB(4,8)save_1%
4070UNTIL t_one(save_1%)=0 OR t_one(1200)
4080save_2%=0
4090REPEAT
4100PRINT£X,t_two(save_2%):save_2%=save_2%+1
4110PRINT TAB(20,8)save_2%
4120UNTIL t_two(save_2%)=0 OR t_two(1200)
4130CLOSE£X
4140ENDPROC
4150:
4160DEFPROCplot
4170CLS
4180REM CREATE GRAPHICS WINDOW
4190VDU 24,30;20;1279;1023
4200REM DRAW AXIS
4210MOVE 0,0
4220PLOT 0,220,170
4230PLOT 1,0,800
4240PLOT 0,0,-800
4250PLOT 1,900,0
4260PLOT 0,-900,0
4270MOVE 220,170
4280FOR x_plot%=0 TO 5:MOVE 900*x_plot%/5+220,170:PLOT 1,0,-20:PLOT 0,0,20:NEXT
4290MOVE 220,130
4300FOR y_plot%=0 TO 5
4310MOVE 220,800*y_plot%/5+170
4320PLOT 1,-20,0
4330PLOT 0,20,0
4340NEXT
```

```
4350:
4360REM X-CO-ORDS
4370MOVE 220,170
4380VDU 5
4390FOR q%=0 TO 5
4400MOVE 900*q%/5+220,130
4410PRINT;q%*DT%*60/5
4420NEXT
4430MOVE 800+370,130:PRINT;"secs"
4440FOR min%=0 TO 5
4450MOVE 900*min%/5+220,70
4460PRINT;min%*DT%/5
4470NEXT
4480MOVE 800+370,70:PRINT;"mins"
4490:
4500REM Y-CO-ORDS
4510MOVE 220,170
4520FOR p%=0 TO 5
4530MOVE 120,800*p%/5+170
4540PRINT;p%*1200/5
4550NEXT
4560MOVE 80,1000:PRINT;"C"
4570MOVE 80,960:PRINT;"O"
4580MOVE 80,920:PRINT;"U"
4590MOVE 80,880:PRINT;"N"
4600MOVE 80,840:PRINT;"T"
4610MOVE 80,800:PRINT;"S"
4620:
4630t_1%=0
4640MOVE 220,170
4650REPEAT
4660DRAW 220+t_one(t_1%)*(900/(DT%*60)),t_1%*800/1200+170
4670t_1%=t_1%+1
4680UNTIL t_one(t_1%)=0 OR t_one(1200)
4690PRINT; "   RAT A"
4700MOVE 0,0
4710:
4720t_2%=0
4730MOVE 220,170
4740REPEAT
4750DRAW 220+t_two(t_2%)*(900/(DT%*60)),t_2%*800/1200+170
4760t_2%=t_2%+1
4770UNTIL t_two(t_2%)=0 OR t_two(1200)
4780PRINT; "   RAT B"
4790MOVE 0,0
4800ENDPROC
4810:
4820END
```

LISTING OF SKINNER BOX ANALYTICAL PROGRAM

```
 10REM ****ANALYSIS PROGRAM FOR DD****
 20:
 30DIMt_one(1600)
 35DIMt_two(1600)
 40:
 50INPUT "Filename to be analysed..."F$
 60MODE131
 70:
 80REM ****COLLECT DATA FROM DISC****
 90X=OPENINF$
100INPUT£X,E$,H$,G$,F$,h%,TM$,FW$,RAT$
110INPUT£X,p%,q%,n%,A$,B$,C$,D$
120INPUT£X,G%,K%,H%,L%,E%,I%,F%,J%
130INPUT£X,M%,N%,O%,P%
140INPUT£X,d%,e%
144FOR i%=0 TO 7:PRINT:NEXT
150INPUT£X,S%,T%,U%,V%
240CLOSE£X
250:
260REM ****SCREEN DISPLAY****
270PRINT TAB(2,1)"Date.... "E$
280PRINT TAB(40,1)"Filename...."F$
290PRINT TAB(40,2)"Training Conditions.... "H$
300PRINT TAB(2,2)"Drug.... "G$
310PRINT TAB(30,3)"BOX No. 1"
320PRINT TAB(10,5)"Rat No. ";p%
330PRINT TAB(30,5)"FR Value ";n%
340PRINT TAB(50,5)"Reward lever "A$
350PRINT TAB(30,14)"BOX No. 2"
360PRINT TAB(10,16)"Rat No. ";q%
370PRINT TAB(30,16)"FR Value ";n%
380PRINT TAB(50,16)"Reward lever "B$
390PRINT TAB(2,7) "REWARD LEVER COUNT = ";E%
400PRINT TAB(2,18) "REWARD LEVER COUNT = ";F%
410PRINT TAB(43,7) "WRONG LEVER COUNT = ";I%
420PRINT TAB(43,18) "WRONG LEVER COUNT = ";J%
430IF t_one(0)>0 THEN PRINT TAB(2,8) "TIME AT LAST REWARD LEVER PRESS= ";
435PRINT TAB(2,8)t_one(E%-1) ELSE
437PRINT TAB(2,8)"TIME AT LAST REWARD LEVER PRESS= 0"
440PRINT TAB(43,8) "WRONG lever count at FRF....";K%
450PRINT TAB(2,9) "FRF value for REWARD lever...";M%
460PRINT TAB(43,9) "FRF value for WRONG lever...";N%
470PRINT TAB(2,10) "Time at FRF value.....";d%
480PRINT TAB(43,10) "FRF value response time.....";E%/h%
490IF t_two(0)>0 THEN PRINT TAB(2,19) "TIME AT LAST REWARD LEVER PRESS= "
495PRINT TAB(2,19)t_two(F%-1) ELSE
497PRINT TAB(2,19) "TIME AT LAST REWARD LEVER PRESS= 0"
500PRINT TAB(43,19) "WRONG lever count at FRF....";L%
510PRINT TAB(2,20) "FRF value for REWARD lever...";O%
520PRINT TAB(43,20) "FRF value for WRONG lever...";P%
530PRINT TAB(2,21) "Time at FRF value.....";e%
540PRINT TAB(43,21) "FRF value response time.....";F%/h%
550PRINT TAB(55,12) "TOTAL COUNTS = ";E%+I%
560PRINT TAB(55,23) "TOTAL COUNTS = ";F%+J%
570PRINT TAB(2,11) "FRF value at end of expt for REWARD lever = ";S%
580PRINT TAB(2,12) "FRF value at end of expt for WRONG  lever = ";T%
590PRINT TAB(2,22) "FRF value at end of expt for REWARD lever = ";U%
600PRINT TAB(2,23) "FRF value at end of expt for WRONG  lever = ";V%
610:
620REM ****PRINTOUT****
630INPUT "DO YOU REQUIRE A PRINTOUT...."P$
640IF P$="Y" THEN PROCprintout ELSE 650
650INPUT "DO YOU REQUIRE A PLOT..."GRAPH$
```

```
652IF GRAPH$="Y" THEN 10000 ELSE 660
660END
670:
680DEFPROCprintout
690:
700VDU2,1,27,1,ASC"E"
710VDU2,1,27,1,ASC"W",1,ASC"1"
720PRINT TAB(7)"LIGHT-DARK DISCRIMINATION"
730PRINT
740PRINT
750VDU2,1,27,1,ASC"W",1,ASC"0"
760PRINT TAB(2)"DATE...";E$ TAB(45)"FILENAME...";F$
770PRINT
780PRINT
790PRINT TAB(2)"Drug...";G$ TAB(40)"CHOSEN RAT = "RAT$
800PRINT TAB(2)"Conditions....";H$ TAB(40)"Start Time..."TM$
810PRINT TAB(2)"Length of session.....";h%"mins"
815PRINT TAB(40)"Food withdrawal..."FW$
820PRINT
830PRINT
840PRINT
850PRINT
860REM ****PRINT-OUT FOR FIRST BOX****
870VDU2,1,27,1,ASC"E"
880VDU2,1,27,1,ASC"W",1,ASC"1"
890PRINT TAB(15)"BOX No. 1"
900VDU2,1,27,1,ASC"W",1,ASC"0"
910PRINT
920PRINT
930PRINT TAB(2)"Rat No. ";p% TAB(25)"FR value = ";n%
935PRINT TAB(48)"REINFORCEMENT lever = ";A$
940PRINT TAB(56)"WRONG lever = ";C$
950PRINT
960PRINT
970PRINT TAB(2) "REWARD-LEVER COUNT = ";E%, TAB(43) "WRONG-LEVER COUNT = ";I%
980PRINT
990PRINT TAB(2) "REWARD lever count at FRF......";G%
995PRINT TAB(43)  "WRONG lever count at FRF......";K%
1000PRINT
1010PRINT TAB(2) "FRF value for REWARD lever.....";M%
1015PRINT TAB(43) "FRF value  for WRONG lever.....";N%
1020PRINT
1030PRINT TAB(2) "Time at FRF value........";d%
1035PRINT TAB(43)"Rate of lever press..."E%/h%
1040PRINT
1050PRINT TAB(14) "FRF value at end of expt for REWARD lever = ";S%
1060PRINT
1070PRINT TAB(14) "FRF value at end of expt for WRONG  lever = ";T%
1080PRINT
1090PRINT
1100PRINT TAB(2) "TOTAL COUNTS = ";E%+I%
1110REM ****PRINT-OUT FOR SECOND BOX****
1120PRINT
1130PRINT
1140PRINT
1150PRINT
1160PRINT
1170PRINT
1180VDU2,1,27,1,ASC"W",1,ASC"1"
1190PRINT TAB(15)"BOX No. 2"
1200VDU2,1,27,1,ASC"W",1,ASC"0"
1210PRINT
1220PRINT
1230PRINT TAB(2)"Rat No. ";q% TAB(25)"FR value = ";n%
1235PRINT TAB(48)"REINFORCEMENT lever = ";B$
```

```
1240PRINT TAB(56)"WRONG lever = ";D$
1250PRINT
1260PRINT
1270PRINT TAB(2) "REWARD-LEVER COUNT = ";F% TAB(43) "WRONG-LEVER COUNT = ";J%
1280PRINT
1290PRINT TAB(2) "REWARD lever count at FRF......";H%
1295PRINT TAB(43) "WRONG lever count at FRF......";L%
1300PRINT
1310PRINT TAB(2) "FRF value for REWARD lever.....";O%
1315PRINT TAB(43) "FRF value for WRONG lever.....";P%
1320PRINT
1330PRINT TAB(2) "Time at FRF value........";e%
1335PRINT TAB(43)"Rate of lever press..."F%/h%
1340PRINT
1350PRINT TAB(14) "FRF value at end of expt for REWARD lever = ";U%
1360PRINT
1370PRINT TAB(14) "FRF value at end of expt for WRONG  lever = ";V%
1380PRINT
1390PRINT
1400PRINT TAB(2) "TOTAL COUNTS = ";F%+J%
1410PRINT
1420PRINT
1430PRINT TAB(2) "COMMENTS...."
1435 FOR r%=0 TO 5:PRINT:NEXT
1440VDU2,1,27,1,ASC"F"
1450VDU 3
1460ENDPROC
1470:
10000:
10020MODE128
10030REM CREATE GRAPHICS WINDOW
10040VDU 24,30;20;1279;1023
10050REM DRAW AXIS
10060MOVE 0,0
10070PLOT 0,220,170
10080PLOT 1,0,800
10090PLOT 0,0,-800
10100PLOT 1,900,0
10110PLOT 0,-900,0
10120MOVE 220,170
10130FOR x_plot%=0 TO 5:MOVE 900*x_plot%/5+220,170:PLOT 1,0,-20:PLOT 0,0,20:NEXT
10140MOVE 220,130
10150FOR y_plot%=0 TO 5
10160MOVE 220,800*y_plot%/5+170
10170PLOT 1,-20,0
10180PLOT 0,20,0
10190NEXT
10200:
10210REM X-CO-ORDS
10220MOVE 220,170
10230VDU 5
10240FOR qq%=0 TO 5
10250MOVE 900*qq%/5+220,130
10260PRINT;qq%*h%*60/5
10270NEXT
10280MOVE 800+370,130:PRINT;"secs"
10290FOR min%=0 TO 5
10300MOVE 900*min%/5+220,70
10310PRINT;min%*h%/5
10320NEXT
10330MOVE 800+370,70:PRINT;"mins"
10340:
10350REM Y-CO-ORDS
10360MOVE 220,170
10370FOR pp%=0 TO 5
10380MOVE 120,800*pp%/5+170
```

```
10390PRINT;pp%*1750/5
10400NEXT
10410MOVE 80,1000:PRINT;"C"
10420MOVE 80,960:PRINT;"O"
10430MOVE 80,920:PRINT;"U"
10440MOVE 80,880:PRINT;"N"
10450MOVE 80,840:PRINT;"T"
10460MOVE 80,800:PRINT;"S"
10470:
10480t_1%=0
10490MOVE 220,170
10500REPEAT
10510DRAW 220+t_one(t_1%)*(900/(h%*60)),t_1%*800/1750+170
10520t_1%=t_1%+1
10530UNTIL t_one(t_1%)=0 OR t_one(1750)
10540PRINT; "   RAT 1A"
10550MOVE 0,0
10560:
10570t_2%=0
10580MOVE 220,170
10590REPEAT
10600DRAW 220+t_two(t_2%)*(900/(h%*60)),t_2%*800/1750+170
10610t_2%=t_2%+1
10620UNTIL t_two(t_2%)=0 OR t_two(1750)
10630PRINT; "   RAT 1B"
10640MOVE 0,0
10650END
```

SAMPLE PRINTOUT OF SKINNER BOX EXPERIMENTAL SESSION

LIGHT–DARK DISCRIMINATION

DATE...19-9-89 FILENAME...E30/R1

Drug...MEL10
Training Conditions....MD Time of expt...2215
Length of session.....5mins Food withdrawal...1400

BOX No. 1

Rat No. 1 FR value = 20 CHOSEN lever = RIGHT
REJECT lever = LEFT

CHOSEN lever count = 460 REJECT lever count = 1

Last CHOSEN lever press = 281.9 REJECT lever count at FRF.....1

FRF value for CHOSEN lever.....95 FRF value for REJECT lever.....5

Time at FRF value.....49.97 Rate of lever press...92

FRF value at end of expt for LEFT lever = 0

FRF value at end of expt for RIGHT lever = 100

TOTAL COUNTS = 461

BOX No. 2

Rat No. 1 FR value = 20 CHOSEN lever = RIGHT
REJECT lever = LEFT

CHOSEN lever count = 366 REJECT lever count = 0

Last CHOSEN lever press = 299.97 REJECT lever count at FRF.....0

FRF value for CHOSEN lever.....100 FRF value for REJECT lever.....0

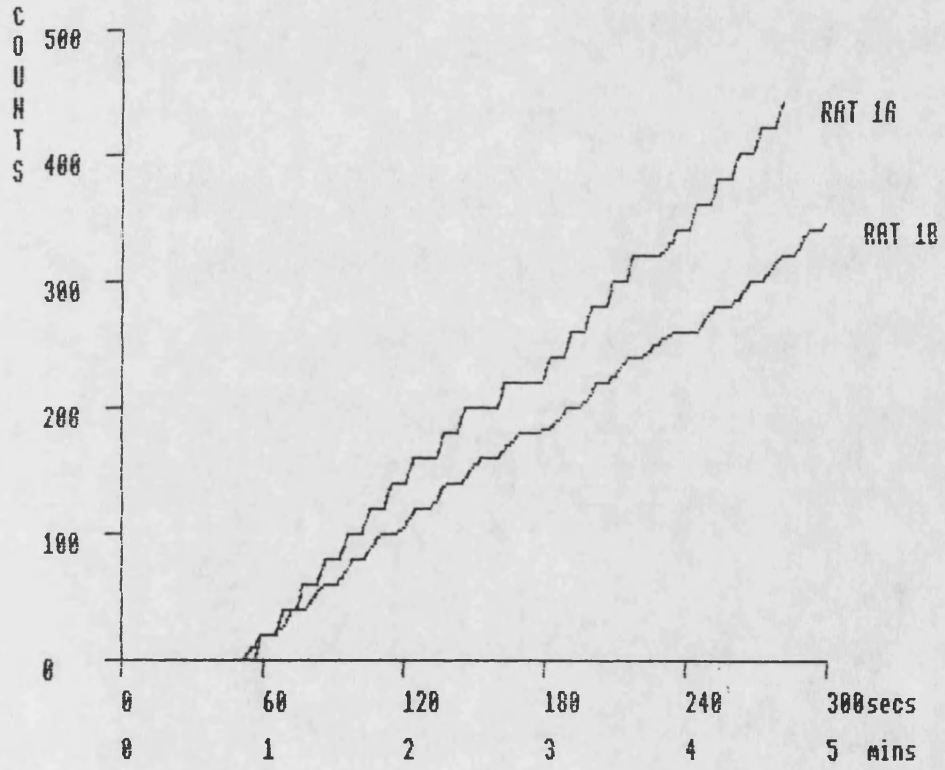
Time at FRF value.....36.86 Rate of lever press...73.2

FRF value at end of expt for LEFT lever = 0

FRF value at end of expt for RIGHT lever = 100

TOTAL COUNTS = 366

CUMULATIVE RESPONSE CURVE



CENTRAL 5-HT RECEPTOR SUBTYPES

Nomenclature	5HT	5HT	5HT	5HT	5HT	5HT	5HT	5HT
Effector pathways	cAMP K+ channel (G-prot)	cAMP	IP_3/DG	cAMP	-	IP_3/DG	cation channel	cAMP
Selective agonists	8-OHDPAT	TFMPP mCPP	TFMPP mCPP	5-CT	-	DOB DOI	2-methyl-5-HT	5-CT
Selective Antagonists	(-)-pind (-)-prop	(-)-pind (-)-prop	ritanserin pizotifen	-	-	ritanserin ketanserin pizotifen	MDL72222 ICS205930	ICS205930
Radioligands	[H]8-OHDPAT	[H]cyan-opindolol [H]5-HT	[H]mesu-lergine	[H]5-HT	[H]5-HT	[H]ketan-serin	[H]zaco-pride [H]ICS-205930	-
Cloned	Yes	-	Yes	-	-	Yes	-	-
Location	Post-S Soma-dendritic	Auto-R Post-S	Post-S	Auto-R Post-S	-	Post-S	-	-
Behavioral functional correlates	"5-HT syndrome" hyperphagia hypothermia	hypophagia	hypo-activity	-	-	head-twitches (in rodents)	-	-

Adapted from: TIPS (1990), 11(1) (Suppl); Dumius et al., (1988); Leonhardt et al., (1989).
Abbreviations: Pind = Pindolol; Prop = Propranolol; Post-S = Post-synaptic; Auto-R = Autoreceptor; cAMP( ) = stimulation of adenylate cyclse; cAMP( ) = inhibition of adenylate cyclase; G-prot = coupled to G-protein; IP_3/DG = stimulation of phospho-metabolism.